Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

SHOUT
SHARE
IT

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 879

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER November 15, 1920

THE MOSAIC DISEASE OF CUCURBITS

By

S. P. DOOLITTLE, Assistant Pathologist
Office of Cotton, Truck, and Forage Crop
Disease Investigations

CONTENTS

	Page
Scope of the Investigations	1
The Mosaic Disease	1
Cause of the Disease	18
Infectious Nature of the Disease	29
Mosaic Transmission	40
Method of Overwintering	47
Control Measures	63
Summary	64
Literature Cited	68

WASHINGTON
GOVERNMENT PRINTING OFFICE
1920

THE INVESTIGATIONS of the cucumber mosaic reported in this paper were begun by the writer in 1914 at Hamilton, Mich., while a graduate student at the Michigan Agricultural College. In 1915 and 1916 they were continued at Big Rapids, Mich., and in 1917–18 at Madison, Wis., under cooperative relations between the Bureau of Plant Industry of the United States Department of Agriculture, the experiment stations of Michigan and Wisconsin, and certain interested pickle growers, under the joint direction of Dr. E. A. Bessey, Dr. L. R. Jones, and Mr. W. W. Gilbert. Laboratory facilities and material were furnished jointly by the cooperating parties and land, tools, and labor by a local pickle company.

The writer wishes especially to thank Messrs. Bessey, Jones, and Gilbert for assistance and supervision throughout the course of the work.

This paper was presented to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy.

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 879

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER November 15, 1920

THE MOSAIC DISEASE OF CUCURBITS.

By S. P. Doolittle, *Assistant Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations.*

CONTENTS.

	Page.		Page.
Scope of the investigations	1	Method of overwintering	47
The mosaic disease	1	Control measures	63
Cause of the disease	18	Summary	64
Infectious nature of the disease	29	Literature cited	68
Mosaic transmission	40		

SCOPE OF THE INVESTIGATIONS.

DURING the last four years the more important diseases of the cucumber have been studied by various workers in cooperation with the Bureau of Plant Industry of the United States Department of Agriculture. One of the most significant facts developed in the early work was the occurrence of a mosaic disease, heretofore practically unrecognized, which is probably at the present time the most widespread and destructive disease of cucumbers. The disease, however, is not confined to the cucumber but affects to a greater or less extent most of the cucurbits grown in this country.

The present bulletin deals with the nature, transmission, and overwintering of the disease in relation to cucurbits. The greater portion of the work has been done with the cucumber as grown for pickling purposes, in an attempt to develop field control measures.

THE MOSAIC DISEASE.

HISTORY.

Although mosaic has been reported in the field and greenhouse for some years, it is only recently that the disease has received detailed attention. Selby (26)[1] in Ohio in 1902 and Stone (29) in Massachusetts in 1909 recorded a mosaic disease on the leaves of greenhouse cucumbers, and Clinton (8) in 1908 noted a chlorosis of muskmelon leaves in Connecticut. It is not certain, however, that all

[1] The serial numbers in parentheses refer to "Literature cited" at the end of the bulletin.

these were true mosaic, as the disease was not proved to be infectious in any case and no mention was made of symptoms on the fruit. Selby (27) stated in 1910 that cucumber mosaic was transmitted like that on tobacco. There is no early record of the disease in the field, but it is certain that it had caused serious losses in the Central States for some time prior to 1914, and from evidence obtained from pickle men in the vicinity of Chicago it is practically certain that the mosaic had been a serious disease in that section for 10 to 15 years and was responsible for forcing at least one pickle company out of business. Ruggles and Stakman (25) in 1911 reported the mosaic as occurring in Minnesota and used the name wart disease in describing it. Coons (10) in 1915 reported that this disease was then causing serious losses in the field in Michigan and had been present in some localities for seven years. He described the chief symptoms and stated that the progress of the disease indicated that it was infectious.

The first proof of the infectious nature of the disease and the methods by which it is transmitted was presented in the papers of Gilbert (14), Jagger (17), and Doolittle (11) in 1916. Jagger (18) later reported the appearance of another type of cucumber mosaic, affecting only the leaves of the plant, and in a further paper (19) mentioned a third type as occurring on Summer Crookneck squash.

Stakman and Tolaas (28) mentioned nubbin or wart disease of cucumber as an infectious disease occurring in both the field and the greenhouse in Minnesota. McClintock (22) in 1916 observed possible cases of transmission of the disease through the seed and described its occurrence in the district near Norfolk, Va. Clinton (9) also gave data as to the severity of the disease in Connecticut.

Freiberg (13) in 1917 reported the disease on various cucurbits in Missouri and described inoculation experiments. Jagger (20) in 1918 also reported numerous cross-inoculations from cucumber to other cucurbits and showed that cucumber mosaic could be transmitted to plants outside the Cucurbitaceæ.

These papers include all the available material on cucumber mosaic to the present, with the exception of an abstract by Doolittle and Gilbert (12) which dealt with certain phases of the work here considered in greater detail.

GEOGRAPHICAL DISTRIBUTION.

The general occurrence of cucurbit mosaic in the field was not recognized until after these studies were begun, but investigation has shown that it is widely distributed on Cucurbitaceæ throughout the United States. Doubtless the extensive cultivation of cucumbers makes it seem more common on that crop, but it may occur on practically all cucurbits in localities where it is found on cucumbers.

The disease is probably most widespread and serious in Wisconsin, Michigan, Indiana, northern Illinois, and on Long Island. It is re-

ported to have caused serious losses in Illinois as early as 1908 and was present about as early in Michigan and Wisconsin. The center of the pickle-growing industry is located in these States, which devote a large acreage to the crop. Here the disease is present to some extent in almost all localities, and in most cases there is infection every year.

The disease occurs commonly in New York and is becoming serious in the seed-growing districts of Ohio and Iowa. It has been found quite prevalent in the fields around Rocky Ford and Greeley, Colo., causes severe losses in the trucking region about Norfolk, Va., and does much injury in many sections of Florida and Louisiana. Recent inspections of the principal city markets of the Western and Pacific Coast States have shown the mosaic disease to be present in practically all of them, and in many cases it was very prevalent. It is also reported in the field from Maine, Massachusetts, Minnesota, Vermont, Connecticut, West Virginia, New Jersey, Pennsylvania, Georgia, Texas, California, Nebraska, and Ontario, Canada.

In the greenhouse it has caused serious injury for several years in Michigan, Wisconsin, Illinois, and New York and has also been reported from Mississippi, Ohio, Indiana, Minnesota, Pennsylvania, Louisiana, and Kansas. Up to the present the disease has been found only in the United States and Canada, but the fact that it has not been reported from other parts of the world may be due to its not having been extensively studied elsewhere.

ECONOMIC IMPORTANCE.

Cucurbit mosaic is the most serious disease of cucumbers in the Middle West, and yearly it increases in severity in districts where this crop is grown on a large scale. The disease is not confined to the cucumber, but causes considerable loss on the muskmelon and to a less extent on squash and pumpkin.

The greater losses on the cucumber are due partly to the special severity of the disease on the fruits of this host, but more especially to the fact that the acreage of cucumbers far exceeds that of any other cucurbit. The increase in the pickling and trucking industries in certain sections has localized much of this crop in restricted areas, and thus furnished conditions very favorable to the spread of such a highly infectious disease. The other cultivated cucurbits ordinarily are grown on a smaller scale, and thus the loss is distributed among many small growers, so that it is less noticeable. Cucumber plants infected with mosaic are practically worthless, owing to the great reduction in yield and to the fact that the fruits produced are so mottled and deformed that they are usually refused by pickling companies and are of little value for market purposes. The disease spreads very rapidly, and many fields may become 50 to 75 per cent diseased almost before picking has begun. As a result of

the losses from mosaic, many growers, especially those in certain sections of the Middle West, have ceased to grow cucumbers, despite increased prices (Pl. I, *C*).

In the case of forcing cucumbers, growers occasionally lose an entire crop, because, in addition to the reduced yield and deformed fruit, mosaic often causes the sudden wilting and death of the plants under glass. An additional factor in the loss occasioned by mosaic is the fact that affected table stock sells for about one-fourth the price obtained for sound fruits, owing entirely to the effect on their appearance.

Of the other cultivated cucurbits squash and muskmelon seem most susceptible to the mosaic, but neither of these plants is injured as severely as the cucumber. The muskmelon vines are stunted and bear only a few fruits, which are inferior in quality but only occasionally show mosaic symptoms. Squashes are similarly affected and in addition sometimes have warty and deformed fruits, the Summer Crookneck variety being most often and most severely attacked. The disease is less common and of minor importance on the pumpkins, gourds, and ornamental cucurbits, which are of less commercial value.

CUCURBIT HOSTS.

Most of the species of the family Cucurbitaceæ appear to be susceptible to mosaic. Field observations have proved that most cultivated species are commonly affected, and that the disease also occurs on wild species. The host range has been further extended by cross-inoculation experiments which included most genera and species of cucurbits found in this country and many varieties of the commoner cultivated species.

Most of the field inoculation tests were made during 1916 and 1917. All inoculated plants were kept under insect-proof cages, and reciprocal inoculations were made in most cases. The various species were usually inoculated in stems or leaves with the expressed juices of mosaic cucumber plants, but many successful inoculations were made between other species, leaving no doubt that the disease is identical on the various cucurbit hosts. From 10 to 50 successful inoculations have been made with all the species noted below.

In the case of cucumber, *Cucumis sativus* L., 15 varieties were inoculated successfully, and all seemed equally susceptible. Mosaic was also produced on the West Indian gherkin, *C. anguria* L., on a closely related variety, *C. grossulariaeformis* Hort., and on *C. metuliferus* Mey.

Inoculations on 11 varieties of muskmelon, *Cucumis melo* L., have all produced the disease, including the pomegranate melon, *C. melo* var. *dudaim* Naudin. Seven varieties of squash and four varieties of pumpkin, *Cucurbita pepo* L., *C. moschata* Duchesne, and *C. maxima* Duchesne, have also been infected.

On the gourds infection occurred on 12 varieties, including *Cucurbita pepo* L., *Luffa cylindrica* Roehm, *L. acutangula* Roxbg., *Lagenaria vulgaris* Ser., and *L. leucantha* Rusby.

Mosaic has also been produced by inoculation on the following species: *Momordica involucrata* E. Meyer, *M. charantia* L., *Ecballium elaterium* A. Rich., *Benincasa hispida* Cog., and *Trichosanthes anguina* L.

The wild species, *Micrampelis lobata* (Michx.) Greene, is common in many parts of the country both as an ornamental vine and growing wild and is often diseased. The identity of this disease with that on the cucumber as well as with that which occurs on the less common wild species, *Sicyos angulatus* L., has been proved by repeated inoculation.

From data so far collected, the watermelon, citron, and other species of Citrullus seem partially resistant to the disease. No successful inoculations have been made by the writer on any plants of this genus with the exception of the green-seeded citron, *Citrullus vulgaris* Schrad., and watermelons grown in the field have never been found infected although often intertwined with mosaic-diseased cucurbits. Jagger (19) reported having obtained a single case of mosaic on watermelon by inoculation, but was not able to repeat such infection. He also noted the disease on the green-seeded citron, but not on the red-seeded variety. Freiberg (13), at the Missouri Botanical Gardens, also reported mosaic on the citron and showed photographs of the disease.

CROSS-INOCULATIONS TO OTHER PLANTS.

INOCULATIONS FROM MOSAIC CUCUMBERS TO PLANTS OF OTHER FAMILIES.

Certain field observations have at times suggested that cucurbit mosaic might possibly be transmitted to plants of other families. An example of such a case occurred at Madison, Wis., in 1916. A few plants of *Martynia louisiana* Mill. appeared in one of the cucumber plats, the seed having been accidentally included with that of the cucumber. A large number of the cucumber plants became infected with mosaic early in the season, and a few of the martynia plants also developed a disease of the mosaic type later in the summer, the circumstances suggesting a possible transference of the infection from the cucumber. Inoculations were made from the mosaic martynia to healthy cucumber plants and also from mosaic cucumber to healthy martynia, but all the inoculations gave negative results.

Most of the inoculations from cucumber to plants outside the Cucurbitaceæ, however, have been with plants known to be subject to infectious mosaic diseases, such as tomato, tobacco, bean, petunia, and pokeweed (*Phytolacca decandra* L.), although a few inoculations have been made on plants subject to unknown chlorotic diseases.

The methods used in this inoculation work were very similar to those used in the other cucumber-mosaic studies. Where field inoculations were made, the plants were protected with insect-proof cages wherever possible, and in the greenhouse all plants used in such inoculations were kept isolated from other mosaic experiments. The inoculum was prepared in much the same way as in other cucumber-mosaic inoculations, either the expressed juice or crushed tissues of mosaic plants being used. Where the juice of a mosaic plant was employed, the parts of the plant used as inoculum were passed through a sterilized food chopper and the juice expressed and filtered through filter paper. Inoculations were made at from three to five points in each of the younger leaves, a drop of the expressed juice being pricked into the leaf with a sterile needle. The stem usually was inoculated at one or more points, a slight incision being made with a sterile scalpel carrying a drop of the inoculum.

TABLE I.—*Record of inoculations from mosaic-diseased cucumber plants to plants of other families.*

Date inoculated.	Plant inoculated.	Preparation of inoculum.	Number of plants inoculated.	Results.	
				Number of mosaic plants.	Date last observed.
Aug. 4, 1916	Tobacco	Expressed juices	3	0	Aug. 17, 1916
Mar. 11, 1917	do	do	4	0	Mar. 26, 1917
Apr. 27, 1917	do	do	4	0	May 28, 1917
May 8, 1917	do	do	8	0	Do.
May 20, 1917	do	Crushed leaf tissues	20	0	June 14, 1917
Oct. 1, 1917	do	do	18	0	Oct. 30, 1917
Nov. 5, 1917	do	Expressed juices	21	0	Dec. 1, 1917
Aug. 4, 1916	Tomato	do	3	0	Aug. 17, 1916
Aug. 18, 1916	do	do	6	0	Aug. 31, 1916
Feb. 23, 1917	do	do	4	0	Mar. 20, 1917
Feb. 30, 1917	do	do	6	0	May 2, 1917
Apr. 5, 1917	do	Crushed leaf tissues	15	0	May 8, 1917
Oct. 8, 1917	do	do	12	0	Nov. 19, 1917
Dec. 8, 1917	do	Expressed juices	15	0	Jan. 12, 1918
Aug. 4, 1916	Wax bean	do	9	0	Aug. 17, 1916
Do	Lima bean	do	6	0	Do.
Feb. 15, 1917	Red kidney bean	do	15	0	Mar. 10, 1917
Mar. 24, 1917	do	do	10	0	Apr. 12, 1917
Sept. 7, 1916	*Martynia louisiana*	do	12	0	Sept. 23, 1916
Aug. 15, 1916	Potato	do	8	0	Do.
Aug. 16, 1915	Pokeweed (*Phytolacca decandra.*)	do	5	0	Sept. 30, 1915
Mar. 22, 1916	Petunia	do	6	0	Apr. 10, 1916
Aug. 1, 1917	*Ambrosia trifida*	do	12	0	Sept. 1, 1917
Aug. 5, 1917	do	do	10	0	Do.

Where the crushed tissues of the mosaic plant were used, a fragment of one of the young leaves was crushed in a sterile dish with sterile instruments and small portions of this crushed material inserted in slight incisions made at one or two points in the stem of the plant inoculated and other pieces sometimes pricked into the young leaves.

The results of all the inoculations from mosaic cucumber to plants outside the cucurbits have been negative,[1] as shown by Table I.

[1] Recent experiments (1919) indicate that cucumber mosaic may be transmitted to *Martynia louisiana* by means of aphids taken from mosaic cucumber plants. Inoculations from martynia plants infected in this manner have also produced the disease on the cucumber.

Jagger (20), however, states that the white-pickle type of mosaic disease, which is the one studied by the writer, has been transmitted to one of the Lobeliaceæ (*Lobelia erinus* L. var. *gracilis*) and to one of the Compositæ (*Helianthus debilis* Nutt.). It is thus evident that in some cases the disease may pass from the cucumber to hosts outside the Cucurbitaceæ.

TABLE II.—*Record of inoculations of cucumber plants with infectious mosaic diseases of noncucurbitaceous plants and with suspected mosaic plants of other families.*

PLANTS HAVING MOSAIC DISEASES KNOWN TO BE INFECTIOUS USED AS SOURCE OF INOCULUM.

Date inoculated.	Plant used as source of inoculum.	Preparation of inoculum.	Number of plants inoculated.	Results.	
				Number of mosaic plants.	Date last observed.
Aug. 25, 1915	Mosaic tomato	Expressed juices	5	0	Sept. 10, 1915
Sept. 3, 1915	do	do	9	0	Sept. 20, 1915
Dec. 21, 1915	do	do	5	0	Jan. 22, 1916
Feb. 4, 1916	do	do	8	0	Feb. 27, 1916
Aug. 19, 1916	do	Crushed leaf tissues	6	0	Sept. 5, 1916
Sept. 7, 1916	do	Expressed juices	8	0	Sept. 23, 1916
Feb. 18, 1917	do	Crushed leaf tissues	8	0	Mar. 3, 1917
Feb. 20, 1917	do	Expressed juices	12	0	Mar. 21, 1917
Dec. 2, 1917	do	do	15	0	Jan. 16, 1918
Feb. 15, 1918	do	Crushed leaf tissues	24	0	Mar. 1, 1918
Dec. 21, 1915	Mosaic tobacco	Expressed juices	5	0	Jan. 22, 1916
Aug. 29, 1916	do	do	6	0	Sept. 23, 1916
Feb. 17, 1917	do	do	6	0	Mar. 18, 1917
Mar. 30, 1917	do	do	18	0	Apr. 20, 1917
May 20, 1917	do	do	20	0	June 14, 1917
Nov. 10, 1917	do	Crushed leaf tissues	35	0	Dec. 1, 1917
Jan. 11, 1918	do	Expressed juices	25	0	Jan. 30, 1918
Sept. 3, 1915	Mosaic bean	do	9	0	Sept. 20, 1915
Sept. 10, 1915	do	do	10	0	Do.
July 18, 1916	do	do	5	0	Aug. 17, 1916
Aug. 30, 1916	do	do	4	0	Sept. 23, 1916
Mar. 5, 1918	do	do	18	0	Mar. 23, 1918
Sept. 3, 1915	Mosaic potato	do	9	0	Sept. 20, 1915
Sept. 10, 1915	do	do	10	0	Sept. 23, 1916
Aug. 23, 1916	do	do	8	0	Do.
Sept. 3, 1915	Mosaic pokeweed (*Phytolacca decandra*).	do	9	0	Sept. 20, 1915
Sept. 10, 1915	do	do	10	0	Do.
Aug. 23, 1916	do	do	6	0	Sept. 15, 1916
Aug. 30, 1916	do	Crushed leaf tissues	4	0	Sept. 23, 1916
Mar. 19, 1916	Mosaic petunia	Expressed juices	5	0	Apr. 1, 1916
Apr. 1, 1916	do	do	8	0	Apr. 22, 1916
Sept. 3, 1915	Peach with yellows (leaves and twigs).	do	3	0	Sept. 20, 1915
Sept. 10, 1915	do	do	17	0	Do.
July 20, 1916	do	do	5	0	Aug. 17, 1916
Sept. 10, 1915	Little peach leaves and twigs.	do	17	0	Sept. 20, 1915
Aug. 30, 1916	do	do	8	0	Sept. 23, 1916

PLANTS HAVING DISEASES OF THE MOSAIC TYPE OF SUSPECTED INFECTIOUS NATURE USED AS SOURCE OF INOCULUM.

Sept. 10, 1915	Mosaic milkweed (*Asclepias syriaca*).	Expressed juices	9	0	Sept. 20, 1915
July 20, 1915	do	do	5	0	Aug. 17, 1916
Aug. 25, 1916	do	do	7	0	Sept. 23, 1916
Sept. 10, 1916	Mosaic red clover (*Trifolium pratense*).	do	10	0	Sept. 20, 1916
July 20, 1916	do	do	5	0	Aug. 17, 1916
Sept. 7, 1916	Mosaic martynia (*Martynia louisiana*)	do	8	0	Sept. 23, 1916
Aug. 10, 1917	do	Crushed leaf tissues	6	0	Sept. 5, 1917
Sept. 8, 1916	Mosaic pepper (*Capsicum annuum*).	Expressed juices	3	0	Sept. 23, 1917
Aug. 18, 1917	Mosaic ragweed (*Ambrosia trifida*).	do	7	0	Sept. 12, 1917
Aug. 30, 1917	do	Crushed leaf tissues	8	0	Sept. 24, 1917
Aug. 30, 1916	Mosaic sumac (*Rhus typhina*).	Expressed juices	8	0	Sept. 23, 1916

INOCULATIONS FROM MOSAIC PLANTS OF OTHER FAMILIES TO CUCUMBER.

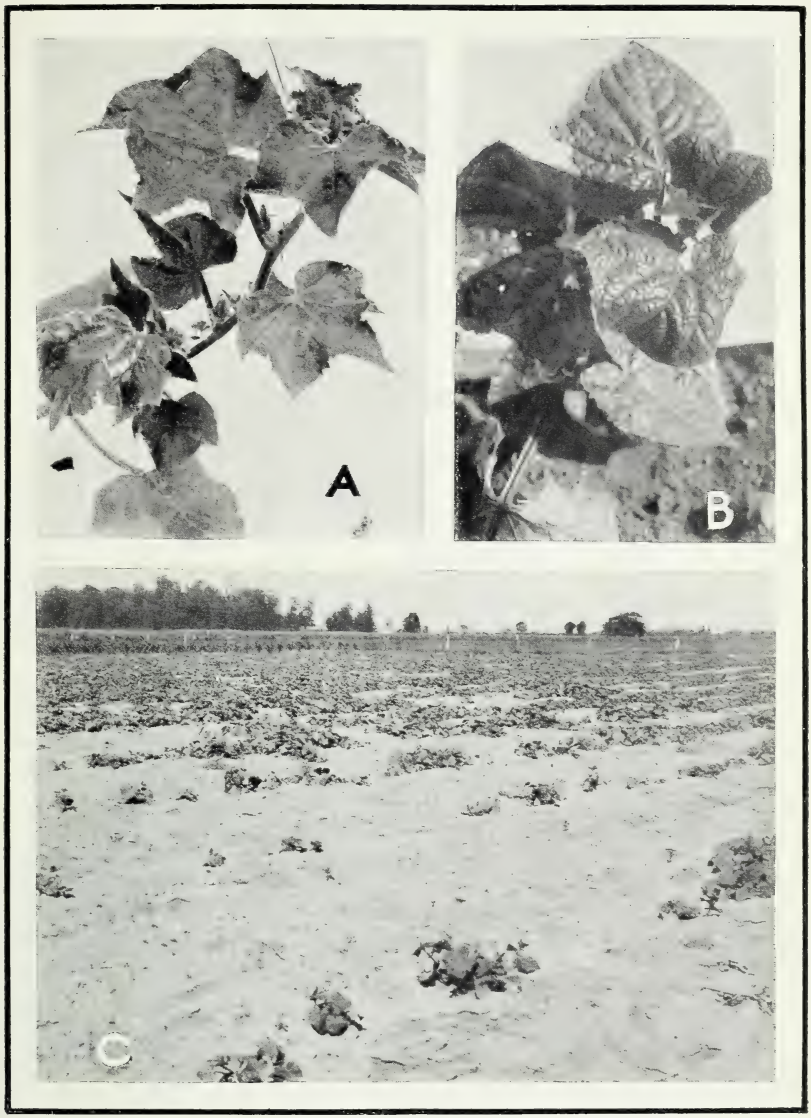
Attempts have also been made to transmit mosaic diseases found on plants outside the Cucurbitaceæ to the cucumber. A considerable number of inoculations have been made from tomato, tobacco, bean, and other plants having infectious mosaic diseases and also from plants which showed diseases of the mosaic type but whose infectious nature was not proved, such as pepper (*Capsicum annuum* L.), sumac (*Rhus typhina* L.), milkweed (*Asclepias syriaca* L.), red clover (*Trifolium pratense* L.), and others. Inoculations in most cases were made with the expressed juice of the mosaic plant in the manner already described. All the results of these inoculations have been negative, as shown in Table II and there is as yet no evidence that any of the diseases on the hosts tried are identical with the mosaic disease on the cucurbits.

MOSAIC SYMPTOMS.

The progress of the mosaic disease in the plant is characterized by distinct morphologic and physiologic changes, which are most marked in the leaves and fruits. These consist principally of an abnormal mosaic coloring, accompanied by certain malformations and dwarfing of growth. The symptoms vary somewhat according to the age of the plant and its vigor of growth at the time of infection, but the general characters are the same in all stages of growth. There is also a variation in the symptoms on the different species of Cucurbitaceæ which are susceptible to the disease. The leaves of different species show many diverse forms of mosaic coloring, and the fruits may or may not be affected, although usually they show marked symptoms soon after the plant becomes diseased. The symptoms on the cucumber have been described in greater detail, as it is the most important host and has been most closely studied, but the general characteristics will apply to all other hosts except for the variations mentioned later.

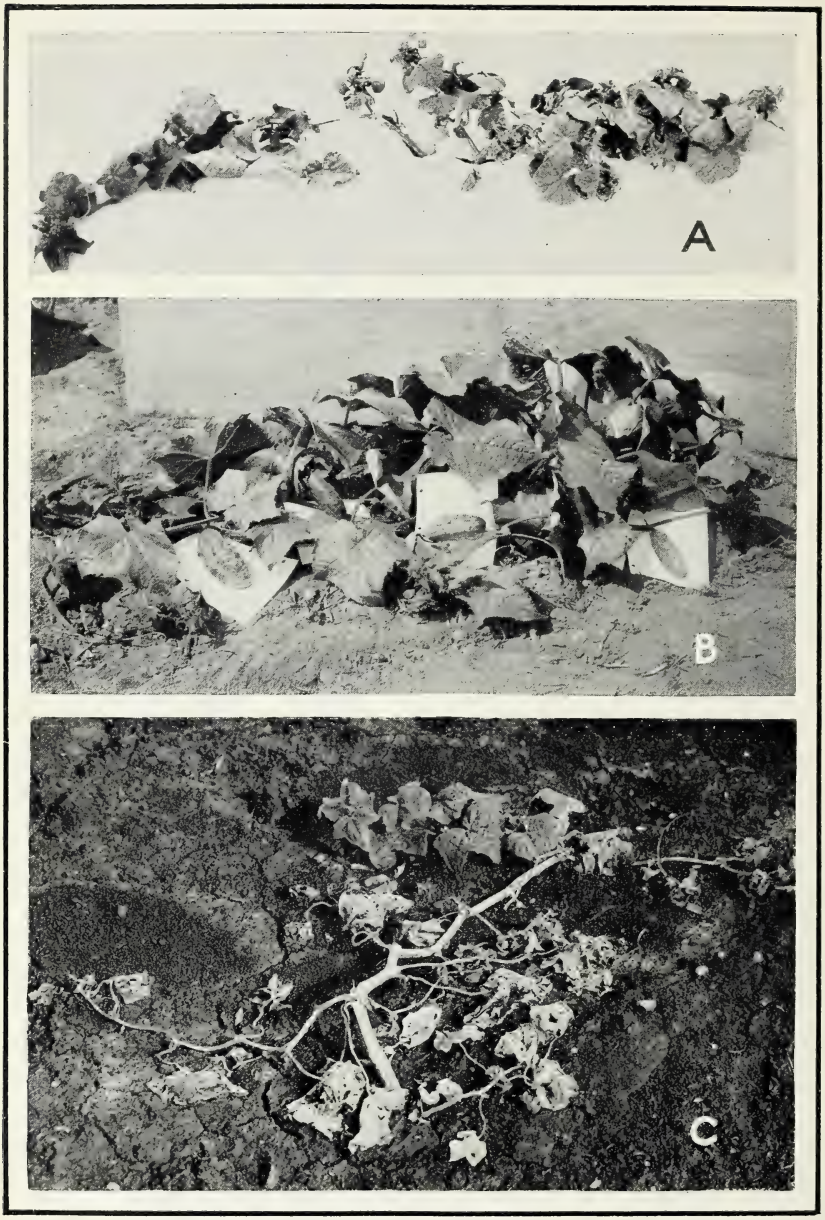
PLANT SYMPTOMS.

Symptoms on affected seedlings.—Seedling cucumbers are rarely affected with mosaic either in the field or in the greenhouse. The disease may appear on seedlings under certain conditions, however, as is shown by the fact that a very general and serious mosaic infection occurred on the first true leaves of cucumber seedlings at Big Rapids, Mich., in the summer of 1918. The seed was planted late in August and when the plants came up the rest of the field was already rather badly infected with the disease. When such infection does occur, the first symptoms appear as a yellowing and wilting of the cotyledons, accompanied by a slight mottling of the young leaves. A sudden checking of growth soon follows and the later leaves remain small, wrinkled, and distorted and have a distinct



MOSAIC ON YOUNG CUCUMBER PLANTS AND A BADLY DISEASED FIELD.

A, A single mosaic plant obtained from seed from mosaic plants in a test at Big Rapids, Mich., 1917; *B*, a young mosaic cucumber plant, showing the curling and savoyed effect on the smaller leaves; Big Rapids, Mich., 1916; *C*, a cucumber field at Holland, Mich., on August 19, 1915, showing severe stunting and loss to the late-planted crop due to infection while the plants were small; the early planted portion in the background was not so badly affected. Photographed by W. W. Gilbert.



MOSAIC CUCUMBER PLANTS.

A, Mosaic cucumber plant infected while young, showing dwarfing and curling of the leaves and shortening of the stems; Greeley, Colo., September, 1917. Photographed by W. W. Gilbert. *B*, Cucumber mosaic from inoculation with aphids from mosaic plants, the fruit symptoms being most marked; Big Rapids, Mich., 1915. Photographed by W. W. Gilbert. *C*, Mosaic plants in late stage, showing death of the basal leaves, whitening of the main stem, and the dwarfing and curling of the tip leaves. Photographed by M. W. Gardner, September, 1916.

mottling of yellowish green. Such plants seldom produce fruit and are usually short lived, rarely reaching a length of more than 12 inches.

Symptoms on plants infected when young.—The period of most general infection begins when the plants are about 6 weeks old and growing vigorously. At this time they have from six to eight leaves and are commencing to run. The first symptoms in all cases appear in the young leaves which are still in the process of development and usually are not easily recognized by an untrained observer. The youngest of the leaves develop small greenish yellow areas, often not more than a millimeter or two in diameter, occasionally circular, but more often limited in outline by the smaller veins of the leaf. These spots are slightly more translucent than the remainder of the leaf and are often scarcely visible except by transmitted light. Less commonly the normal green of the leaf changes to a peculiar yellow at the tip and the sharply defined yellow areas do not appear. Accompanying these symptoms there is a gradual downward curling of the edges of the leaf, and the surface presents a finely wrinkled appearance, the tissue between the small veins becoming slightly raised so as to form minute convex surfaces (Pl. I, *B*.) Following these early symptoms, the gross mosaic characters develop rapidly, and the leaves become strikingly mottled with green and yellow. Such leaves are wrinkled and savoyed in appearance and may be somewhat distorted and curled (Pl. II, *A*).

All growth subsequent to infection is much dwarfed, the stem internodes are shortened, the leaves attain only about one-half normal size, and the petioles are reduced in length. As the plant becomes older the wrinkled and savoyed character of the leaf is more marked, but the mottling is generally less conspicuous.

Plants infected at this stage blossom sparingly and set few fruits. They send out few runners and have a bunched and bushy habit of growth, with the leaves lying close to the ground in a rosettelike clump.

Symptoms on plants infected when nearly mature.—In the case of large plants the general symptoms are similar to those described above. The first signs of the disease appear in the youngest leaves, where the yellowing and curling before noted are the usual indications of infection, while all the older leaves appear normal and may remain so for some time. In some cases, however, the youngest leaves turn yellow and wilt, their edges becoming brown and withered, and all the leaves of a shoot may thus wilt within a few days. Ordinarily, however, only three or four leaves at the growing point are affected in this way, and the runner soon sends out a new shoot just back of the withered tip, which shows the ordinary mosaic symptoms. Symptoms may also appear very early on the young fruits, and in the first stages of the disease they are often more marked than those which occur on the leaves (Pl. II, *B*). Frequently the most pronounced

leaf symptoms appear on new secondary shoots put out from the axils of the leaves near the base of the plants. The wilt symptoms are often noted on the cucumber and pumpkin and occasionally on the Summer Crookneck squash and muskmelon.

In the greenhouse this type of response is much more intense, and it is common to have the vine suddenly develop a yellowing and wilting, which extends to the entire plant within two to three days, the older leaves usually showing yellowing but wilting very rapidly. This wilting and death of the plant occur only occasionally in the field and never progress so rapidly there as they do in the greenhouse.

Aside from these differences, the older plants in the field show the same symptoms as plants infected at an earlier stage in their development. The dwarfing and wrinkling of the younger leaves are very pronounced and the mottling is of the same type as that on younger plants. The shortening of the stem internodes and the petioles gives the runners a flattened appearance, and the leaves lie close to the ground.

The older leaves of mature plants are later more severely affected, and the greater portion gradually die off. Portions of the leaf turn yellow, those at the base of the stem being affected first. This may occur as a gradual yellowing of the entire leaf, or more characteristically as a V-shaped yellowed area along one of the large lateral veins of one of the basal lobes. The tissues of these yellowed portions rapidly turn brown and die, beginning at the edges in the angle formed by the lobes and working toward the base of the lateral veins. (Pl. III.) The entire leaf eventually becomes yellow, this yellowing gradually extending to the leaves farther up the stem. Such leaves finally wither and die, leaving the basal portion of the stem bare. This is a characteristic symptom in late stages of the disease, and by the end of the season many plants show 1 to 3 or more feet of bare stem with the dead leaves still attached, but terminating in a cluster of dwarfed, dark-green, mottled, and wrinkled leaves which lie close to the ground. (Pl. II, *C*.)

LEAF SYMPTOMS.

The pattern of coloring which the leaves of mosaic plants assume varies with the species, the age of the plant at the time of infection, and the stage of development of the individual leaves.

Leaf symptoms on cucumbers.—On the cucumber (*Cucumis sativus*) the mosaic mottling is usually most pronounced and typical on the young leaves of plants which are growing rapidly (Pl. IV, *A*). The green portions of these leaves are very dark in color and are raised slightly above the surrounding surface, thus giving the leaf its savoyed appearance. In most cases the green areas predominate, being intermingled with spots of light greenish yellow. These yellow spots are irregular in outline and are limited by the small veins (Pl. IV, *B*).

In some cases the green and yellow areas are both small and about equal in number (Pl. III), being scattered irregularly over the leaf and sometimes coalescing to form larger areas, with the green portions rather sharply defined, owing to their raised character. More often, however, there are a few isolated patches of yellow from 3 to 5 millimeters in diameter, the greater portion of the leaf being dark green with the typical savoyed character. On other leaves the yellowing may take the form of large blotches which tend to follow the large veins, the margins not being sharply defined but blending gradually into the green portions of the leaf. In such leaves the savoyed appearance is much less marked.

In the case of older plants the wrinkling of the young leaves is usually very noticeable, but there is less contrast in color. The raised parts of the leaf are dark green, while the remainder is merely a lighter shade of green than that found in normal leaves, the borders of these light spots not being sharply defined. In such plants the base of the leaf will occasionally develop a sharply contrasted mosaic mottling, while the tips of the leaves will remain normal in color for some time.

In the older leaves the symptoms are somewhat different in nature. The savoyed character does not appear in many cases, and the yellowing tends to include the entire leaf in a short time. In leaves which are nearly full grown the yellowing very often extends along the network of the larger veins of one of the basal lobes. The portions of the leaf inclosed within these veins may remain green for some time and have a slightly convex appearance. A pronounced drooping or curling of the leaf often follows in the parts where such a mottling occurs, producing a peculiar wilted appearance in the lobe of the leaf affected.

In other cases certain portions of the leaf turn yellow and the remainder retains its green color for a considerable time. The yellow portions in such leaves often include a few small, isolated, dark-green areas, producing an odd, checkered appearance in these portions of the leaf. This is quite distinct from the ordinary mosaic mottling in that the green tissues show no tendency to be elevated above the surrounding leaf surface. This type of yellowing occurs most commonly in the angle formed by the junction of the large veins at the base of the leaf, but also appears at the tips of the lobes. In such leaves the green portions retain their color for some time. One of the most common symptoms in the older leaves of mature plants commences as a yellowing which appears along the large lateral veins of one of the basal lobes, extending their entire length. These yellow areas are widest at the edge of the leaf and narrow toward the juncture of the veins, producing V-shaped areas which have already been described (Pl. III).

Another symptom on the older leaves which is more common in the greenhouse than in the field, consists of a yellowing which affects nearly all the leaf at the same time. In such leaves the tissues adjoining the larger veins retain their color longest and outline these veins against the yellow of the remainder of the leaf. These symptoms are usually associated with the wilting type of the disease.

Leaf symptoms on muskmelons.—The leaf symptoms on the muskmelon (*Cucumis melo*) are very similar to those on the cucumber. The first signs of the disease appear on the youngest leaves, which turn light yellow in color and develop a sharp downward curl. Later, all the younger leaves of the plant show typical mosaic characters (Pl. V, *B*). The light yellow portions of the leaf are of irregular outline and about the same size and color as those on the young leaves of the cucumber. The dark areas are more definitely outlined on the muskmelon, and the leaves show a very pronounced curling. The characteristic symptoms found on the older leaves of the cucumber are much less common on the muskmelon. The older leaves gradually become yellow, but show little or none of the tendency to die early which appears in mosaic cucumber leaves of the same age.

Leaf symptoms on squashes.—In the case of the squash (*Cucurbita* spp.) the symptoms of the disease are much the same on all varieties. The younger leaves usually develop an extremely savoyed appearance, the darker parts of the leaf being much more definitely raised above the leaf surface than in the case of the cucumber. The light spots on the leaf are pale yellowish green and tend to coalesce and form a few large blotches rather than the smaller and more numerous spots found on the cucumber (Pl. VI, *A*). The older leaves often become yellow and gradually die off, as in the case of the cucumber, but in many plants these leaves will retain their normal color for some time, the symptoms of the disease being confined to the younger leaves. The most marked symptoms have been found on the Summer Crookneck and Cocozelle bush varieties (*Cucurbita pepo* var. *condensa*), those of the Hubbard type (*Cucurbita maxima*) showing the disease in a less extreme form.

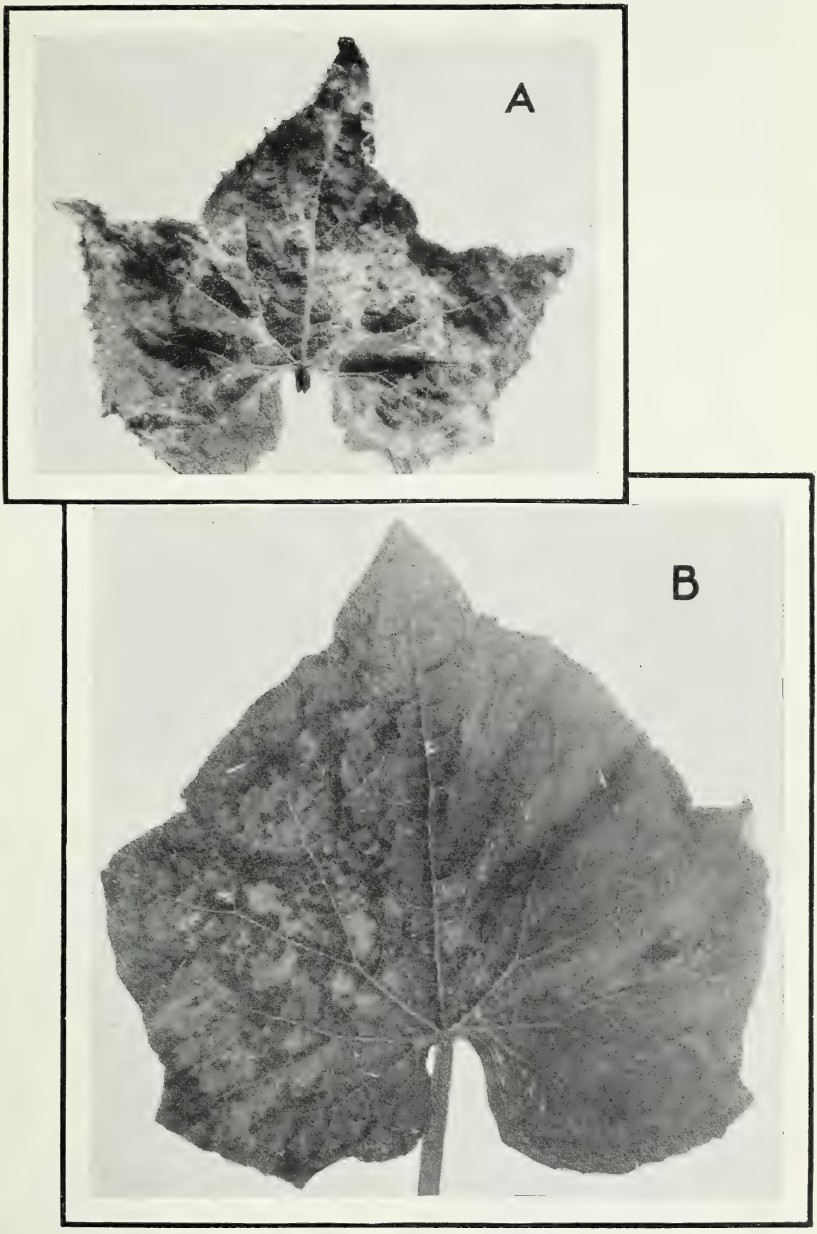
Leaf symptoms on pumpkins.—On the pumpkin (*Cucurbita pepo*) the leaf symptoms are very nearly identical with those found on the squash. The younger leaves develop with the same extreme mottled and wrinkled appearance, the general color of the leaf being a lighter yellow than that of a normal plant. On the pumpkin, however, the older leaves very often show a rapid yellowing and wilting which corresponds very closely to that found on the cucumber in the greenhouse.

Leaf symptoms on other cucurbits.—The general symptoms on the gourds and other ornamental cucurbits are very similar to those on the squash. The chief points of difference occur in the patterns of the mottled leaves. In the case of the Cucurbita gourds the surface

A Hoen & Co Baltimore.

MOSAIC LEAVES, FRUITS, AND BRANCH OF CUCUMBER.

Three fruits illustrating common types of mosaic effect; two leaves, the smaller showing pronounced mottling, common to young leaves, the larger showing dying of older leaves on plants infected when nearly mature; tip of plant with dwarfed and dying leaves.



MOSAIC CUCUMBER LEAVES.

A, Small cucumber leaf, showing extreme mottling and wrinkling characteristic of a severe mosaic attack; Madison, Wis., 1919. *B*, Cucumber leaf, showing simple mottling often seen in the earlier stages of mosaic attack; Big Rapids, Mich., 1915. Photographed by W. W. Gilbert.

of the leaf is finely wrinkled and the yellow areas are very small but present in large numbers, so that the leaf appears to be covered with fine dots of yellow, which in some cases bear a close resemblance to insect injury. This type of mosaic symptoms is also common on the Luffa and Lagenaria gourds, the spots in the latter species being larger and very nearly circular in form. (Pl. V, *A*.)

On the wild cucumber (*Micrampelis lobata*) there is usually a marked variegation and deformity in the leaves of mosaic plants. In most cases the symptoms are similar to those on the cucumber with the exception that the lighter colored portions of the leaf predominate. These are a light yellowish green with an occasional almost colorless area which is in sharp contrast to the surrounding tissue. The other parts of the leaf are a deep green and are raised above the surrounding surface. As a result the savoyed character is usually pronounced, and the leaves are deeply wrinkled and curled. There is also considerable dwarfing and deformity in mosaic leaves, the lobes being irregular in size and outline. In some leaves, however, the difference in color in the various parts of the leaf is less marked, and in such cases the savoyed character does not appear, although the leaf may be distorted in shape. (See Pl. VII, *A*, *B*, and *C*, for comparison.)

While the above types of symptoms occur most commonly, there are often cases in which the greater part of the leaf remains almost a normal green but develops numerous small circular areas of light yellow which are similar to those noted on the Cucurbita gourds. These yellow spots are about 1 millimeter in diameter and are slightly depressed. In other leaves we find a number of small, dark-green areas scattered over the surface, the center of each of them being composed of a small yellow spot similar to those above described. The green portion of these areas is raised, while the yellow centers are slightly sunken, producing a peculiar pitted appearance.

The older leaves of mosaic plants gradually turn yellow, some of them showing the V-shaped yellow patches which occur on the older leaves of the cucumber. This yellowing of the leaves eventually affects most of the plant and is a distinguishing character of the later stages of the disease.

The symptoms on the other wild host, *Sicyos angulatus*, are much like those on micrampelis. The mottling of the young leaves is seldom extreme, but the older leaves tend to yellow and die off rapidly, the dead leaves remaining attached to the stem as in the case of the cucumber.

FRUIT SYMPTOMS.

Fruit symptoms on cucumbers.—The symptoms on the fruits of the cucumber are extremely marked in most cases and when present make the disease easily recognized. The stem end of the young

fruit first becomes mottled with yellowish green, this mottled character gradually spreading over the entire fruit. As this progresses the body of the fruit ordinarily becomes a light yellowish green, intermingled with spots of a much darker green color. These dark portions are usually raised above the surrounding surface in such a way that they form wartlike projections and often produce more or less distortion of the fruit.

The number and size of these protuberances vary greatly, and we find many types of affected fruits (Pl. VIII). In some cases, particularly in the greenhouse, the mottled character is very marked, the fruit being covered with mingled blotches of light and dark green, but with little or no wart production or distortion of shape. Such fruits are common in the field, as also those in which the stem end is the only part to show a well-defined mottling.

More commonly, however, the dark-green portions of the surface are distinctly raised, the projections varying from 1 millimeter up to 2 centimeters in diameter and from 1 to 6 millimeters in height. The general appearance of all such fruits is the same, but the minor characters are subject to great variation. In some cases the warts are small, sharply outlined, isolated, and scattered over the surface in varying numbers (Pl. VIII, *A* to *C*). On other fruits they are larger, coalesce to some extent, and form irregular raised patches, which are sometimes slightly elevated and do not greatly affect the shape of the fruit, while in still other specimens they are so large as to produce an extremely rough and irregular form (Pl. VIII, *D*, *E*). In some cases there may be one or two large warts, often at the stem end, the remainder of the fruit being yellowish white or mottled with yellowish green, but nearly as smooth as normal.

In general, however, the fruits are decidedly mottled in appearance, and show swellings of all sizes, some isolated and others merging into one another in such a way as to produce a very irregular form, the symptoms often being well defined on fruits which are but an inch in length. The fruits of this type have given rise to the names "wart disease" and "nubbin," which have been applied to the disease by growers in some parts of the country.

In the later stages of the disease the vines occasionally produce fruits which are smooth, pale whitish green in color, and rather more blunt at the ends than normal fruits of the same age. In most cases these fruits are mottled with fine spots of yellowish green, and a few dark-green projections appear here and there on the surface. (Pl. VIII.) These are usually small, but occasionally fruits are found which have a single large dark-green swelling near the stem end, producing a most unusual appearance.

These white fruits are responsible for the older common name of the disease, "white pickle," which was the term applied to the trouble

by the growers in Michigan and Wisconsin for some years. The more common green and distorted fruits were classed with the imperfect types which are often produced under unfavorable soil or climatic conditions, as the name "nubbin" in use in Minnesota indicates. All these names, however, neglect the mottled character of both leaves and fruits, which is the symptom most typical of the disease. Since the term mosaic had already been applied to a disease of tobacco and other plants in which the symptoms and general character were much like those on the cucumber, it seemed best to adopt the same name in this case as being more descriptive of the real nature of the disease than those already in use by the growers.

On vines which are old and stunted another type of fruit often occurs which is abnormally dark green, with little or no mottling. Such fruits have a smooth surface, but are much distorted and swollen in appearance and are found only at the end of the season on vines which have nearly ceased to set fruit. Vines occasionally appear to recover from the effects of the disease and toward the end of the season produce fruits with no visible mosaic symptoms.

Many mosaic fruits show a tendency to become russeted while still green, and in the case of the whitish fruits before described this tendency is very marked. As they turn yellow and ripen, the fruits which show less marked mosaic characters may be almost indistinguishable from those of healthy plants, but in general they retain a misshapen and dwarfed appearance. Seed is usually produced, but in the case of fruits set during the later stages of the disease, the seed is slow to mature and often small and shrunken. Mosaic fruits may also have a slightly bitter flavor, particularly those from plants which have had the disease for some time, but this is not a common symptom.

Fruit symptoms on other cucurbits.—Although the leaves of all the cucurbits susceptible to mosaic show symptoms which have the same general character, the fruits in some cases show no evidence of the disease aside from a reduction in size. In general, however, the fruits of other mosaic cucurbits have the same mottled and warty appearance that is found on those of the cucumber, although these symptoms are often less marked or almost obscured as the fruit reaches maturity.

The young fruits of mosaic muskmelon plants are often mottled, and a few dark-green warts may appear, but as the fruits mature the symptoms of the disease become less pronounced and the fruits are nearly normal in appearance. In the case of the Hubbard squash the young fruits of mosaic plants are sometimes mottled and lighter in color than those of healthy plants, but these symptoms are apparently obscured at maturity by the natural wartiness of the fruit. On the Summer Crookneck squash, however, the fruits are

distinctly mottled, and the warty character is even more pronounced than on the cucumber. The symptoms differ from those on most cucurbits, however, in that the raised portions of the fruit are lighter in color than the surrounding surface. The contrast is often very pronounced, the warts being bright orange-yellow and the remainder a dark green (Pl. VI, *B*, *C*).

The fruits of the Large Cheese pumpkin (*Cucurbita moschata*) show very marked mosaic symptoms, but these symptoms have not as yet been noted on any other variety of pumpkin susceptible to the disease. The young fruits are distinctly mottled with light yellow, and the surface is covered with large dark-green warts. (Pl. V, *C*.) At maturity the fruits are irregular in form, deeply furrowed, and the warty character is very pronounced.

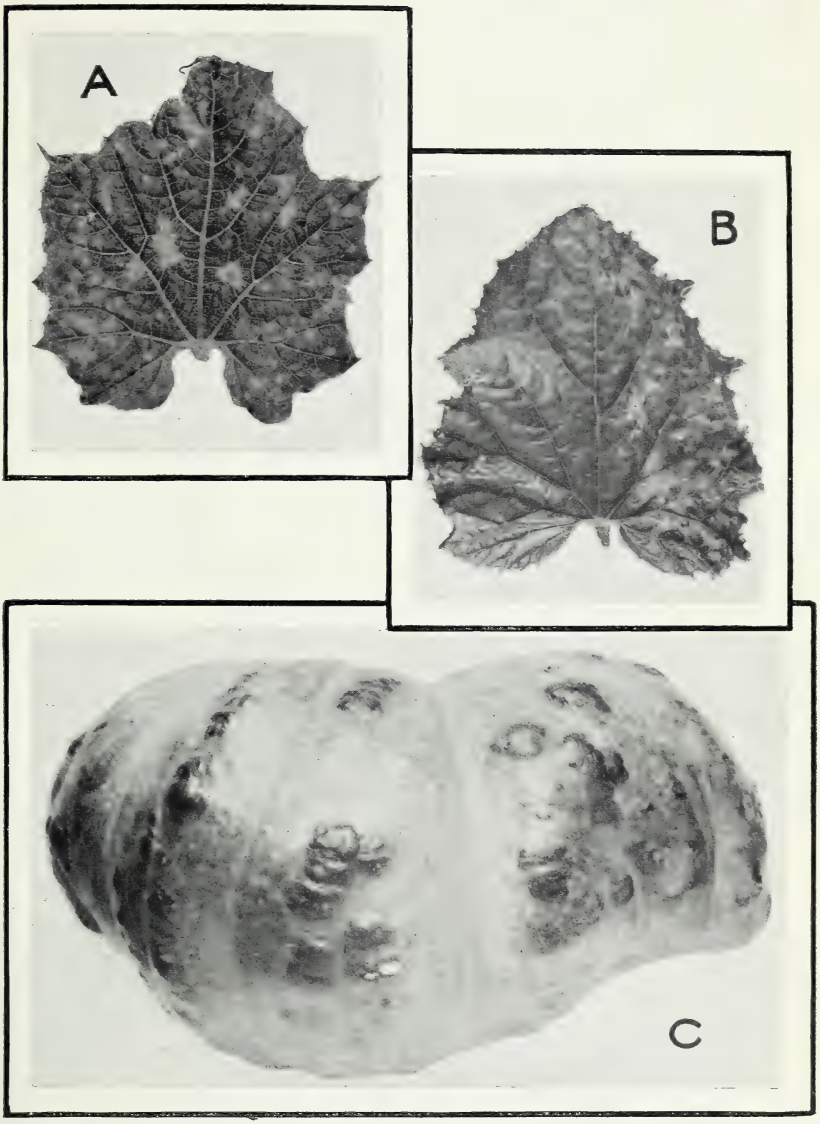
The fruits of most of the squashes, gourds, and the ornamental cucurbits seem to be little affected by the disease, and on many of these hosts no symptoms have as yet been noted. The fruits of the mosaic wild cucumber (*Micrampelis lobata*), however, are usually dwarfed, distorted in shape, and smoother than the fruits of normal plants, the spines being fewer in number and scattered unevenly over the surface. In a few cases fruits have been noted which developed rather large irregular swellings. The epidermis of the fruit eventually splits open at these points and the tissues below push through to form dark-green swollen areas, which are somewhat similar to those found on the cucumber. (See Pl. VII, *D* and *F*.) These symptoms apparently do not occur on all the mosaic fruits of this host, but they have been noted on a number of plants. The fruits of the 1-seeded bur cucumber (*Sicyos angulatus*), on the other hand, do not seem to show any evidence of the disease.

BLOSSOM SYMPTOMS.

Mosaic cucumber flowers are not streaked or variegated as are those of tobacco plants affected with mosaic. They are greatly reduced in number, however, especially the pistillate flowers. Blossoms produced in the later stages of the disease are dwarfed, the corolla often measuring not over three-fourths of an inch in diameter, and are slightly paler than normal blossoms. On other cucurbits the symptoms are of the same character, though the dwarfing is usually less pronounced except in the case of the muskmelon, the blossoms of which are usually reduced in size and number and are a much lighter yellow than those of normal plants.

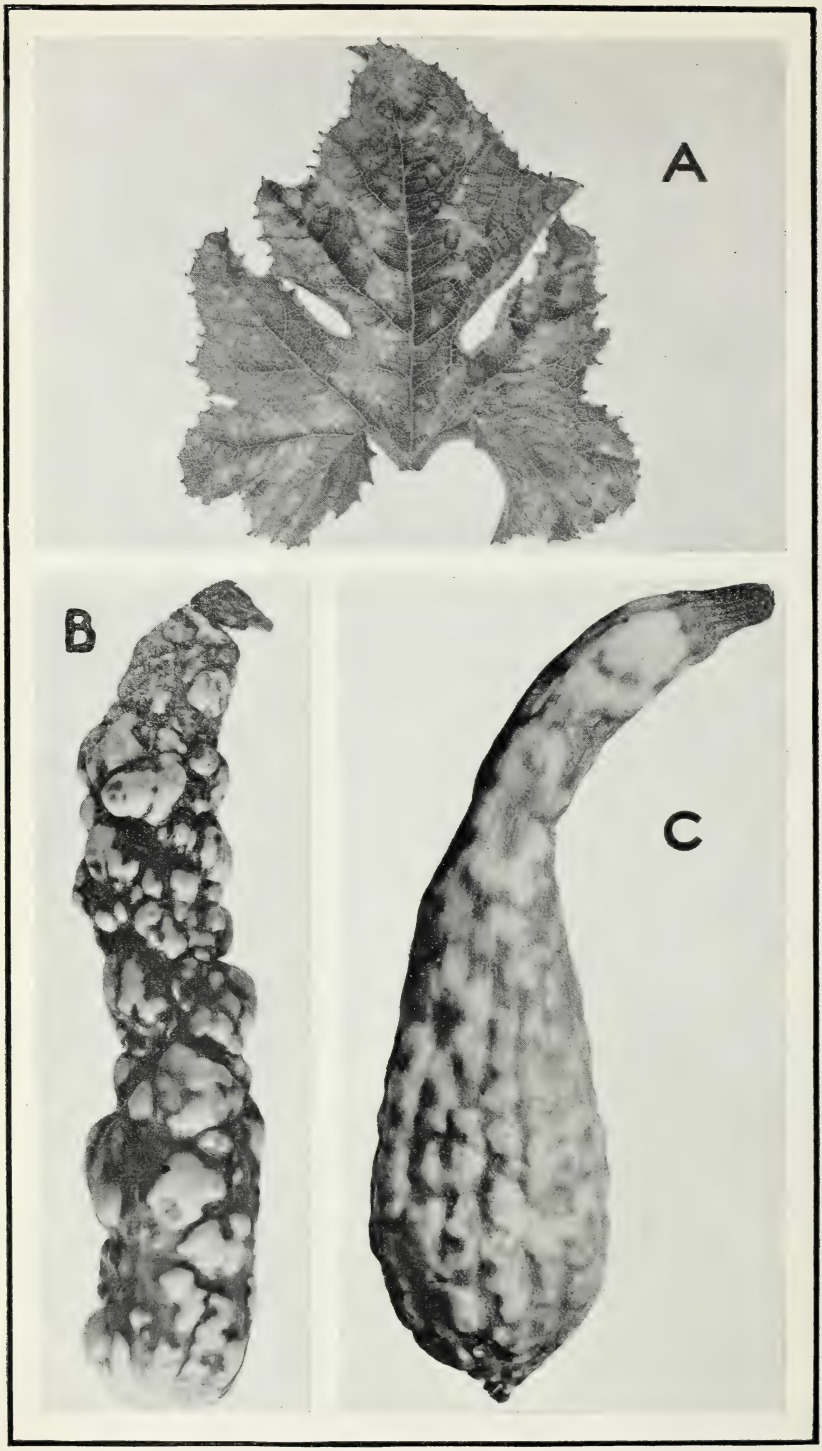
STEM SYMPTOMS.

The stems in the case of the cucumber are shortened, as noted above (Pl. II, *A*), and where the older leaves have died the epidermis



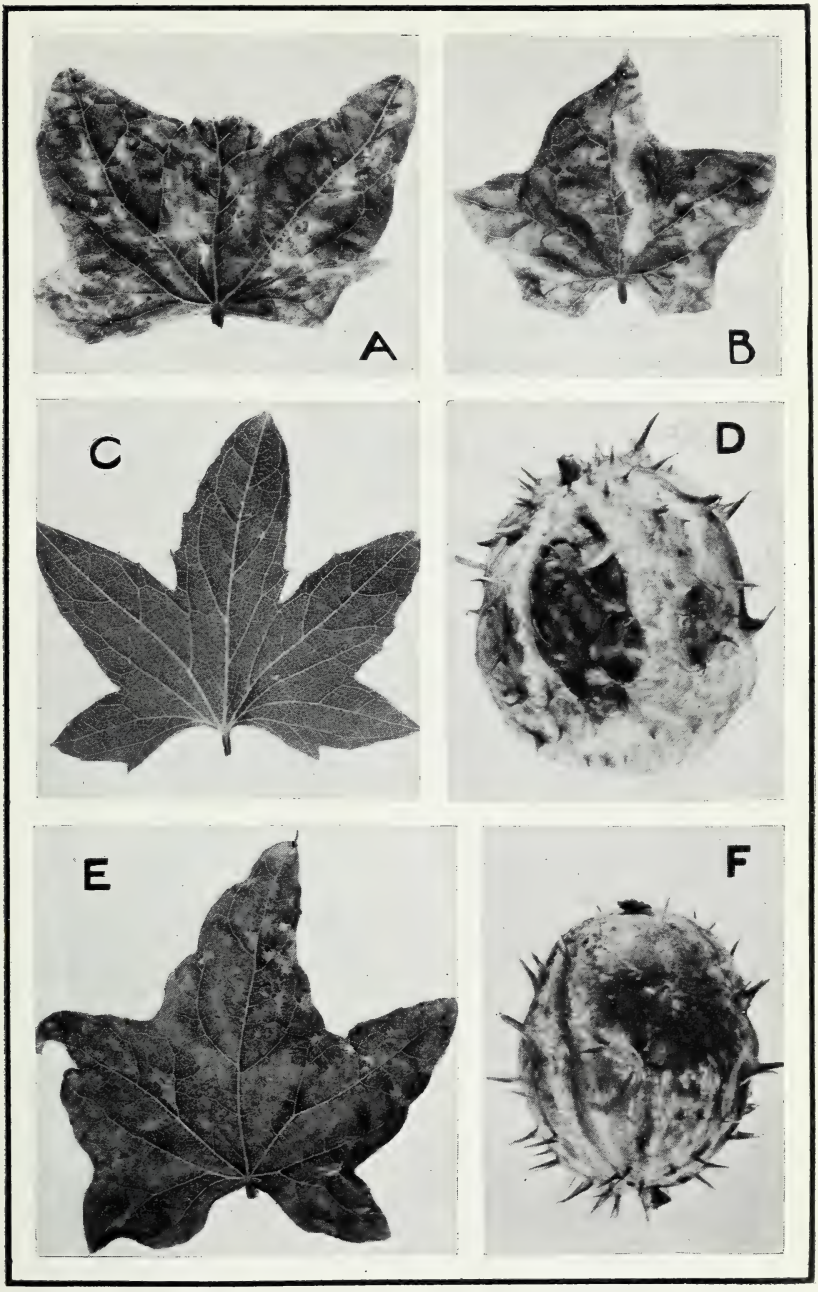
MOSAIC MELON AND GOURD LEAVES AND A PUMPKIN.

A, Mosaic leaf of a Lagenaria gourd, inoculated from cucumber; Madison, Wis., 1917. *B*, Mosaic leaf of a cantaloupe, natural infection; Big Rapids, Mich., 1917. Photographed by W. W. Gilbert. *C*, Mosaic fruit of pumpkin, showing large dark-green swellings on a yellow background; Madison, Wis., 1919.



LEAF AND FRUITS OF MOSAIC SUMMER CROOKNECK SQUASH.

A, Leaf of mosaic Summer Crookneck squash, Madison, Wis., 1917. *B* and *C*, Young fruits of mosaic Summer Crookneck squash exhibiting pronounced symptoms of the disease; *B*, from Irondequoit, N. Y. (photographed by W. W. Gilbert), and *C*, from Madison, Wis., 1917.



MOSAIC WILD CUCUMBER LEAVES AND FRUITS.

A, *B*, and *E*, Mosaic leaves of *Micrampelis lobata*, showing marked mottling, malformation, and wrinkling. Compare with *C*. *C*, Healthy normal leaf of *Micrampelis lobata*, for comparison with mosaic leaves. *D* and *F*, Fruits of *Micrampelis lobata* from a mosaic plant, showing the irregular shape and the wartlike protuberances combined with the splitting of the outer integument; Madison, Wis.

MOSAIC CUCUMBER FRUITS.

A, Small cucumber showing typical "white-pickle" symptoms, the color being yellowish white with a few small raised islands of normal green. *B* and *C*, Fruits with green wartlike protuberances larger than in *A* and more numerous. *D* and *E*, Half-grown fruits with several large dark-green warts and numerous small ones, the rest of the fruit being yellow to greenish white. (*A* to *D*, photographed at Madison, Wis., and *E*, at Washington, D. C.)

becomes whitish gray in appearance (Pl. II, *C*.). This leafless and whitened condition of the basal parts of the stem is one of the most easily recognized symptoms in the late stages of the disease on the cucumber. Such stems seem more brittle than those of normal plants and break more easily. The whitening is due apparently to the loss of leaves and resultant exposure to the weather, since it occurs on healthy plants if the lower leaves are removed. The cucumber stem is not mottled, but it often develops a yellowish green color, somewhat lighter than the dark green of normal stems. Some cucurbits, particularly the squash and pumpkin, occasionally show a slightly mottled appearance of the stem in addition to the shortening of the internodes found on all cucurbits. This mottling consists of pale-green circular patches, about a centimeter in diameter, which occasionally have a slightly sunken appearance.

ROOT SYMPTOMS.

The roots of mosaic cucumber plants show no external evidence of the disease except in its later stages, when the number of finer rootlets is much reduced and the larger roots tend to show a faint yellow color accompanied by an unusual brittleness. Other cucurbits examined have shown no differences between the roots of healthy and diseased plants except for a reduction in the number of finer rootlets.

PATHOLOGICAL ANATOMY.

ROOTS AND STEMS.

Neither the roots nor stems of mosaic cucurbits, so far as examined, show any internal symptoms of the disease. There are no lesions, and sections of these parts have the same appearance as those of normal root and stem tissues.

LEAVES.

The leaves of mosaic cucurbits exhibit many variations in their external symptoms, the size of the yellow areas varying with different hosts, but the internal symptoms are essentially the same in all cases. The dark-green portions are slightly thicker than the normal leaf tissue, and this fact accounts for the blistered and distorted appearance which is very marked in the leaves of cucumbers and squashes. The intervening yellow areas are somewhat thinner than the green portions, but are of nearly the same thickness as the tissue of normal leaves.

When stained sections of the leaf are examined, the palisade cells of the green areas are found to be crowded closely together and to be somewhat narrower and longer than those of the normal leaf. The palisade cells of the yellowed portion, on the other hand, are more

nearly isodiametric and less in number per unit area than in the normal leaf.

The spongy parenchyma of these yellowed areas is also more compact and the intercellular spaces smaller than in the green portions.

The chloroplasts of the cells of the yellowed portions are decidedly smaller than in normal cells and are often pressed so closely to the walls of the cell as to be almost invisible. In contrast to this the chloroplasts in the dark-green areas are so large and numerous as to seem to fill the cells. The vascular elements of mosaic leaves show little variation from the normal.

FRUITS.

On the fruits, the dark-green warty growths and the abnormally light yellowish green of the surrounding surface are the chief external symptoms. Here, the cells directly beneath the epidermis assume the same abnormalities that occur in the palisade cells of the diseased leaves. In the raised green areas, these cells are slightly longer and narrower than in normal fruits, and the chloroplasts are larger and crowded closely together. In the yellow portions of the diseased fruits these subepidermal cells are more nearly square in longitudinal section and their chloroplasts are much reduced in size.

The other tissues of the fruit, including the vascular elements, appear to be normal, the raised character of the green tissues being evidently a result of hyperplasia rather than hypertrophy.

CAUSE OF THE DISEASE.

The cause of cucurbit mosaic, like that of all other infectious chlorotic diseases, is unknown. While the infectious character of the trouble is readily proved, there is no definite indication of the nature of the infective principle or its origin.

RELATION TO SOIL AND WEATHER CONDITIONS.

Extended observations on the mosaic of cucumbers in the field have shown that the physical or chemical nature of the soil apparently has no relation to the origin of the disease. Mosaic will develop with equal rapidity on clay, muck, or sandy soils, and applications of lime, wood ashes, stable manure, or commercial fertilizers have little appreciable effect. It has also been impossible to connect environmental conditions with the disease in any way which indicates that it originates from any unfavorable condition of soil, temperature, or humidity.[1]

[1] Recent investigations indicate that environmental conditions, particularly temperature, may affect the susceptibility of the plant to mosaic, and it is therefore probable that the rapidity with which the disease spreads is partially determined by the conditions of environment.

NO VISIBLE CAUSAL ORGANISM DISCOVERED.

CULTURAL STUDIES.

No parasitic organism has been found to be constantly associated with mosaic plants, although many cultures have been made from roots, stems, leaves, and fruits. In attempting to isolate a causal organism a great variety of media of all types, both vegetable and synthetic, were employed, particular use being made of those containing the juices of the cucumber plant itself. Tissue cultures from all parts of the plant remained sterile in most cases, and where growth occurred it resulted only from contaminations, no single organism being constantly present. Variations in temperature and oxygen supply and in acidity of the media were also tried, but these experiments were equally unsuccessful.

The possibility of an increase of the virus in the culture medium without visible growth has been tested in many cases by inoculation from the medium itself, but no infection has ever appeared. The juices of mosaic plants may produce infection after passing through a Berkefeld filter, and attempts were therefore made to make cultures from these filtrates on various media, but without success. Similar filtrates of the juices of healthy plants when inoculated from infectious filtrates have never shown evidence that the virus could increase on such a medium. All attempts to cultivate the virus from the filtered juice of mosaic plants are also complicated by the fact that the juice loses its infectious character within two to three days after it is expressed from the tissues.

ABSENCE OF ORGANISM IN DISEASED TISSUES.

The absence of a visible causal organism is further supported by the fact that stained sections made from all parts of mosaic plants have given no indication of the presence of any parasite in the tissues. No differences have been detected between the stems and roots of healthy plants and those affected with the mosaic disease. The leaves and fruits show some morphologic differences, but no lesions of any sort are present and no visible organisms have been found in either the fruit or leaves of mosaic plants. All data so far collected, therefore, indicate that the disease is highly infectious, but no causal factor can as yet be associated with infection. On the other hand, the expressed juices of mosaic plants possess definite properties related to their power of infection.

NATURE AND PROPERTIES OF THE MOSAIC VIRUS.

The study of the nature of the infective principle of mosaic diseases has been most extensive in the case of tobacco, many workers having contributed to a knowledge of the properties and character of the virus concerned. While the writer's experiments with the

virus of the mosaic of cucurbits have been less extensive, they indicate that it is similar in many respects to that of tobacco and other plants. The power of infection may be destroyed in each case and is definitely connected with temperature, moisture, age, and other factors.

EFFECT OF HEAT ON THE POWER OF INFECTION.

The expressed juices of mosaic cucumber plants lose their power of infection when heated above 70° C. This temperature has proved the limit in all experiments, as is shown in Table III. In these tests the plant juices were expressed and filtered through filter paper before use. Small test tubes were used and 5 c. c. portions of the juice taken to insure rapid heating to the desired temperature. A constant-temperature water bath was used and the tubes heated 10 minutes and then immediately cooled to room temperature. Inoculations were made from the different tubes to healthy cucumber plants, inoculating into the stem, at the tip and base, and also into two of the young leaves. These plants were protected from possible outside infection by covering them with insect-proof cages.

TABLE III.—*Effect of heat on power of infection of expressed juices of mosaic cucumber plants.*

Date.	Temperature (°C.).	Number of plants inoculated.	Results.	
			Number of mosaic plants.	Date last observed.
Field tests:				
Sept. 3, 1916	45	3	2	Sept. 18, 1916
Do	50	3	2	Do.
Do	55	3	3	Do.
Do	60	3	0	Do.
Do	65	3	2	Do.
Do	70	3	2	Do.
Do	75	3	0	Do.
Do	80	3	0	Do.
Do	Unheated.	3	3	Do.
Aug. 9, 1917	60	4	3	Aug. 21, 1917
Do	65	4	2	Do.
Do	70	4	4	Do.
Do	75	4	0	Do.
Do	80	4	0	Do.
Do	85	4	0	Do.
Do	90	4	0	Do.
Do	95	4	0	Do.
Do	Unheated.	4	4	Do.
Greenhouse tests:				
Oct. 30, 1917	55	6	4	Nov. 10, 1917
Do	60	6	5	Do.
Do	65	6	2	Do.
Do	70	6	3	Do.
Do	75	6	0	Do.
Do	80	6	0	Do.
Do	85	6	0	Do.
Do	90	6	0	Do.
Do	Unheated.	6	4	Do.

EFFECT OF AGE ON THE POWER OF INFECTION.

The expressed juices of mosaic cucumber and other cucurbitaceous plants retain the power of infection for only a short period after their extraction. Repeated tests have shown that the juices of mosaic plants of cucumber, squash, pumpkin, muskmelon, and *Micrampelis lobata* are never infectious for more than three to five days and in most cases lose their virulence within 24 to 48 hours. This is in sharp contrast with results secured by Allard (4) with the juices of mosaic

tobacco plants, which often remain infectious for more than a year, even without the addition of preservatives. In the case of cucurbit mosaic the juices undergo rapid fermentation unless a preservative is added, but various chemicals, such as chloroform, ether, toluene, and glycerine, used at different strengths have never served to increase the time during which the juices remained infectious. Low temperatures have only a slight effect in prolonging the power of infection, and filtrates from Berkefeld filters in which no visible bacterial growth occurs are equally short lived in this regard. The preservatives used with the unfiltered juices were also tried with Berkefeld filtrates, but neither chemical preservatives nor low temperatures have any noticeable effect in prolonging their power of infection.

EFFECT OF DRYING ON THE VIRUS.

As the juices of mosaic plants rapidly lose their power of infection with age, it would be expected that drying would also remove this infective character. This has been the case with the tissues of mosaic cucumber plants. The leaves of mosaic plants when dried at room temperature for periods of 10 days to 1 year have always failed to produce the disease. In these experiments the dried leaf tissues were pulverized and allowed to stand from 8 to 24 hours in a small quantity of sterile distilled water. The water extract was then filtered off and inoculations made both by the injection of the water extract into the stem and leaves of healthy plants and also by the insertion of the ground and moistened leaf tissue in wounds in the stems of healthy cucumber plants. A total of 49 inoculations have been made by these methods, but no infection has ever occurred.

In addition to inoculations with dried leaf tissue, the dried stems, small fruits, and roots of mosaic plants were used in the same way as inocula in 38 inoculations, but always with negative results. The expressed juices of mosaic plants also lose their power of infection on drying. Experiments were made in which these juices were dried either on glass or on filter paper at room temperature. At intervals of seven days the dried material was taken up in sterile distilled water and the water extract pricked into the stems and leaves of healthy cucumber plants. Ten plants were inoculated from the dried material on glass and also from that on filter paper at the end of every seven days, the experiment being continued for five weeks, but no infection resulted from any of the inoculations. The result of experiments on soil transmission of the disease (p. 48) as well as the above data all indicate the rapid loss of the infective principle in the dried tissues of mosaic plants.

EFFECT OF DILUTION ON THE VIRUS.

The power of the virus of cucurbit mosaic to increase rapidly after its injection into the host tissue has been indicated by the rapidity of its distribution through the tissues of the plant inoculated. Further evidence of this rapid increase appears in the results

of inoculations with diluted juices of mosaic cucumber plants. Allard (2) showed that the virus of tobacco mosaic could be diluted to 1:1,000 without reducing its virulence. He also made a number of successful inoculations with the dilutions of 1:10,000, although higher dilutions rarely gave infection. The work of the writer with cucumber mosaic has given similar results, as is shown in Table IV. The juices of mosaic plants were expressed, filtered through filter paper, diluted with sterile distilled water up to 1:100,000, and used for inoculating healthy plants. These experiments show that dilutions of 1:1,000 are as potent as undiluted solutions, but while infections may result from those of 1:10,000 they have never taken place at higher dilutions. In all of this work inoculations were made at the base of the stem and in two or three of the younger leaves, the latter being inoculated at two or three points by pricking a drop of the solution into the leaf. Stems were cut slightly and the cut surface was covered with a drop of the diluted virus.

TABLE IV.—*Effect of dilution with sterile distilled water on the power of infection of the expressed juices of mosaic cucumber plants.*

Date inoculated.	Dilution of expressed juices.	Number of plants inoculated.	Results.	
			Number of mosaic plants.	Disease noted (date).
Series I:				
Apr. 8, 1916	Undiluted	5	3	Apr. 20, 1916
Do	1:100	5	3	Do.
Do	1:1,000	5	3	Do.
Do	1:10,000	5	2	Do.
Do	1:100,000	5	0	
Series II:				
Sept. 8, 1916	Undiluted	4	3	Sept. 18, 1916
Do	1:100	4	3	Do.
Do	1:1,000	4	2	Do.
Do	1:10,000	4	0	
Do	1:100,000	4	0	
Series III:				
May 15, 1917	Undiluted	8	5	May 23, 1916
Do	1:100	8	6	Do.
Do	1:1,000	8	4	Do.
Do	1:10,000	8	2	Do.
Do	1:100,000	8	0	

The regularity of infection which follows inoculation with dilutions of 1:1,000, together with the numerous infections at 1:10,000, shows an apparent power of rapid increase in the infectious material present in mosaic plants, since in these cases the period of incubation was no longer than when the undiluted juices were used.

EFFECT OF VARIOUS CHEMICALS ON THE VIRUS.

Experiments with the effect of various chemicals on the virus of cucurbit mosaic show that the power of infection is easily destroyed by common disinfectants and antiseptics. In this work the juices of mosaic cucumber plants were expressed and filtered through filter paper. The extract was put into test tubes in 5 c. c. portions and

the desired chemical added in such amount and strength that when combined with 5 c. c. of the plant juices it gave the desired concentration. The tubes were then shaken well and allowed to stand for 12 hours. Inoculations were made with these inocula, as in the case of the dilution experiments, into both the stems and leaves of healthy plants. The results shown in Table V indicate that formaldehyde, phenol, mercuric chlorid, and copper sulphate in dilute concentrations were all toxic to the mosaic virus.

The tests with copper sulphate gave definite mosaic infection in one case, but the writer is of the opinion that this is not an indication that the juices are resistant to its action, since the same and weaker dilutions have destroyed the virus in all the other tests. The results in this single case, however, were apparently not due to outside infection, as the plants were caged from the beginning of the season. Chloroform in a 10 per cent mixture destroys the activity of the virus, but a 5 per cent chloroform mixture seems to be harmless. Toluene in a 10 per cent mixture has no apparent effect. In the case of these chemicals special care was used to mix the solution thoroughly, and the tubes were kept tightly corked until inoculations were made. The 10 per cent solution represents a large excess and the 5 per cent a slight excess beyond the possibilities of absorption by the plant juices. The results of these tests have been consistent in most respects, however, as is shown in Table V, and indicate that the virus is not resistant to ordinary disinfectants.

TABLE V.—*Effect of chemicals on the power of infection of the expressed juices of mosaic cucumber plants.*

Date inoculated.	Chemical and strength used.	Number of plants inoculated.	Results.	
			Number of mosaic plants.	Date observed.
Series I (field experiments):				
Aug. 10, 1917	Formaldehyde 1 per cent	4	0	Aug. 20, 1917.
Do	Phenol 1 per cent	4	0	Do.
Do	$CuSO_4$ 1 per cent	4	3	Do.
Do	$HgCl_2$ 1:1,000	4	0	Do.
Do	Chloroform 10 per cent	4	0	Do.
Do	Toluene 10 per cent	4	3	Do.
Do	Untreated juice	4	4	Do.
Do	Distilled H_2O (control)	4	0	Do.
Series II (greenhouse experiments):				
Oct. 23, 1917	Formaldehyde 1 per cent	5	0	Oct. 31, 1917.
Do	Phenol 1 per cent	5	0	Do.
Do	$CuSO_4$ 1 per cent	5	0	Do.
Do	$HgCl_2$ 1:1,000	5	0	Do.
Do	Chloroform 10 per cent	5	0	Do.
Do	Toluene 10 per cent	5	3	Do.
Do	Untreated juice	5	3	Do.
Do	Distilled H_2O (control)	5	0	Do.
Series III (greenhouse experiments):				
Mar. 28, 1918	Formaldehyde 0.5 per cent	7	0	Apr. 20, 1918.
Do	Phenol 0.5 per cent	7	0	Do.
Do	$CuSO_4$ 0.5 per cent	7	0	Do.
Do	$HgCl_2$ 1:2,000	7	0	Do.
Do	Chloroform 5 per cent	7	2	Do.
Do	Toluene 5 per cent	7	4	Do.
Do	Untreated juice	7	5	Do.
Do	Distilled H_2O (control)	7	0	Do.

CHEMICALS AS VIRUS DISINFECTANTS FOR THE HANDS.

In addition to the work with chemicals in direct combination with mosaic juices, further tests were made to determine the value of various solutions as disinfectants for the hands after handling mosaic plants. In these tests the hands were smeared with the juices of a mosaic cucumber plant and then rinsed in the solution to be tested. The healthy plants were then handled in such a way as to bring the hands in contact with slight wounds, the fruits present were picked, small shoots were pinched off, and other wounds made. Formaldehyde, copper sulphate, phenol, and mercuric chlorid were all used in weak solutions, as shown in Table VI, and in addition a strong soap solution and a mere rinsing of the hands in distilled water were tried.

TABLE VI.—*Value of various chemicals as hand disinfectants after handling mosaic cucumber plants.*

Date inoculated.	Chemicals and strengths used as disinfectants.	Number of plants handled.	Results.	
			Number of mosaic plants.	Date observed.
Aug. 17, 1916	Formaldehyde 1 per cent	5	0	Aug. 26, 1916
Do	Formaldehyde 2 per cent	5	0	Do.
Do	Phenol 2 per cent	5	0	Do.
Do	Phenol 5 per cent	5	0	Do.
Do	$HgCl_2$ 1:1,000	5	0	Do.
Do	Soap and water	5	0	Do.
Do	Distilled water	5	0	Do.
Do	Untreated mosaic juices	5	5	Do.
Aug. 18, 1917	Formaldehyde 1 per cent	6	0	Aug. 28, 1917
Do	Phenol 1 per cent	6	0	Do.
Do	$CuSO_4$ 1 per cent	6	0	Do.
Do	$HgCl_2$ 1:1,000	6	0	Do.
Do	Soap and water	6	0	Do.
Do	Distilled water	6	0	Do.
Do	Untreated mosaic juices	6	4	Do.

As no infection occurred after any of these treatments, it is probable that the dilution effect is as important as that of chemical action. Hands covered with the juices of mosaic plants, however, gave a high percentage of infection when no wash was used.

EFFECT OF FILTRATION ON THE VIRUS.

Berkefeld filter.—The presence of a filterable virus as the causal factor in cucurbit mosaic has already been demonstrated. Both Jagger (17) and the writer (11) have shown that the juices of mosaic cucumber plants retain their power of infection after passing through a Berkefeld filter.

The work on this phase of the problem has been continued by the writer, using both the Berkefeld and the Chamberland types of filters.

In the tests with the Berkefeld filter the juices of mosaic cucumbers were expressed and passed through double filter paper. After this

filtration the juices were at once passed through a sterile normal Berkefeld bougie, the bougie, receiving flask, and all connections having been previously sterilized with steam for 45 minutes at 15 pounds' pressure.

Owing to the large amount of finely divided material which remains in suspension in the expressed juices, the filtration process is very slow and with the Berkefeld filter 12 to 14 hours are necessary to obtain 200 c. c. of filtrate from a bougie 1 by 6 inches. The filtrate was removed to sterile test tubes or small flasks by means of sterile pipettes and inoculation made from each lot to beef bouillon, Tubes which proved free from bacteria were used for inoculation. inoculations being made with the unfiltered juice at the same time. These were made by pricking the filtrate into the young leaves at several points and into a wound at the base of the stem. The results given in Table VII show that the filtration of the infectious juices did not destroy their power of infection, most of the filtrate-inoculated plants developing mosaic symptoms as rapidly as those inoculated with the unfiltered juices.

TABLE VII.—*Effect of filtration through a Berkefeld (normal) filter on the infectivity of the expressed juice of mosaic cucumber plants.*

Date inoculated.	Treatment.	Number of plants inoculated.	Results.	
			Number of mosaic plants.	Date last observed.
Aug. 31, 1915	Filtered mosaic juice	6	4	Sept. 15, 1915
Do	Unfiltered mosaic juice	6	5	Do.
Do	Unfiltered healthy juice (control)	6	0	Do.
Sept. 6, 1916	Filtered mosaic juice	8	5	Sept. 14, 1916
Do	Unfiltered mosaic juice	7	5	Do.
Do	Unfiltered healthy juice (control)	8	0	Do.
May 12, 1917	Filtered mosaic juice	8	3	May 21, 1917
Do	Unfiltered mosaic juice	8	6	Do.
Do	Unfiltered healthy juice (control)	8	0	Do.
May 18, 1917	Filtered mosaic juice	10	5	May 31, 1917
Do	Unfiltered mosaic juice	10	6	Do.
Aug. 30, 1917	Filtered mosaic juice	5	2	Sept. 12, 1917
Do	Unfiltered mosaic juice	5	3	Do.
Do	Unfiltered healthy juice (control)	5	0	Do.

Chamberland filter.—In contrast to the results with the Berkefeld filter, the Chamberland type of porcelain bougie has given filtrates that were noninfectious. A considerable number of inoculations have been made, using the filtrates from different types of Chamberland filters, but no positive results have been obtained.

The earlier work was done with filters of the "F" and "B" types about 1 by 8 inches in size, the "B" type being supposedly the finer. Later a set of smaller bougies was obtained which gave a gradation in porosity. These were five-eighths by 6 inches and were graded as L2, L3, L5, L7, L9, and L11. The L2 and L3 grades were supposed to be permeable to the more minute forms of bacteria, and the other

types were supposedly of sufficient density to prevent the passage of visible organisms.

It was hoped that a comparison of the filtrates from these different grades of filters would perhaps indicate the size of the particles responsible for infection, but the results did not warrant any conclusion along this line, as no infection has occurred in a total of more than 100 plants inoculated with these filtrates. The filters of the Chamberland type, being of porcelain, are all denser than the Berkefeld, and filtration is therefore much slower. There is a possibility, therefore, that the infective principle may be held back by absorption, as the suspended material in the plant juices rapidly clogs the filter and covers its surface with a gelatinous layer.

Other filters.—Allard (4) reports that the juices of mosaic tobacco plants become noninfectious if passed through a Livingston atmometer. A filter made of a layer of powdered talc from seven-eighths to 1½ inches thick also gave a noninfectious filtrate. Similar talc filters have been used in experiments with cucumber mosaic, the layer of talc varying from three-fourths of an inch to 1 inch in thickness. Three trials have been made with these filters, and a total of 37 plants have been inoculated from the filtrates. The results have been negative in all cases, however, and it is evident that the filtrate is rendered noninfectious, as in the case of tobacco.

Iwanowski (16), Koning (21), and Beijerinck (6) have stated that the juices of mosaic tobacco plants do not lose their infectious nature when passed through Berkefeld and Chamberland filters, though Iwanowski found that only the first portion of the Chamberland filtrate was infectious. It is thus evident that the infective principle of both tobacco and cucumber mosaic is of such a nature that it can be removed by filters of the finer types.

ENZYMS IN RELATION TO THE DISEASE.

Since many writers have advanced the theory that enzyms, particularly oxidases, are in some way connected with the mosaic of tobacco, a few attempts were made by the writer to isolate a possible causal enzym from the juices of mosaic cucumber plants. The fact that the juices of such plants usually lose their power of infection within 24 to 48 hours has proved an almost insurmountable obstacle in such work, and so little has been done that it is not feasible to draw any definite conclusions. Tests of the juices of healthy and diseased plants which have been passed through filter paper have shown the presence of oxidases and peroxidases in both cases, the guaiacum reaction being used. Very little difference in the intensity was noted, but the juices of diseased plants seem to show a slightly stronger reaction for both enzyms. Similar tests of both healthy and mosaic plant juices after passage through a Berkefeld filter have shown a weaker test for both oxidase and peroxidase in all cases,

the intensity in healthy and diseased juices after filtration being approximately equal. Attempts have been made to precipitate an enzym with 45, 70, and 80 per cent alcohol, using the juices of mosaic plants filtered through filter paper. The precipitates gave peroxidase reactions in all cases, but inoculations made with solutions of these precipitates were never successful.

COMPARISON OF CUCURBIT MOSAIC WITH TOBACCO MOSAIC.

The properties of the virus of cucumber mosaic are strikingly like those attributed to the virus of tobacco mosaic. As already indicated, the symptoms of the two diseases are very similar, as are also the changes produced in the anatomy of the respective hosts. Also in both hosts the points at which successful inoculations can be made correspond with one exception, i. e., in tobacco root inoculations produce infection. The vigor of growth and age of the plants are important factors in the infection of both hosts. Insects and the handling of mosaic and healthy plants in pruning and picking are responsible for much of the field transmission of tobacco as well as cucumber mosaic. The first symptoms appear in the young leaves, both in tobacco and cucumbers, although the virus is found in all parts of the stem and leaves, regardless of the development of mosaic symptoms. In tobacco the virus is present in the flower parts, including the placental column and integument of the ovule, as is shown by Allard (3). It is also present in the mature seeds, according to the same writer. In the case of the cucumber, the virus is present in the flower parts and in the immature fruits, but has never been found in the mature seed. The vascular system may be concerned in the distribution of the virus in both cases, at least in the writer's opinion, since the evidence on which the theory is based is much the same for both hosts.

The work on the properties and nature of the virus of tobacco mosaic has been much more extensive than that on cucumber, and furnishes a valuable basis for comparison. The thermal death point as worked out by Beijerinck (6), Woods (32), Iwanowski (16), and later by Allard (4) seems to lie between 80° and 100° C. This is somewhat higher than that of cucumber mosaic, which is rendered nonpathogenic if heated above 70° C. The expressed juices of mosaic tobacco plants will retain their power of infection for a year, or more, in some cases, whether preservatives are used or not, and the dried leaves will remain infectious for relatively long periods. This is different from the disease on cucumbers, where the expressed juices are seldom infectious for more than 48 hours and the dried tissues have never proved infectious.

Disinfectants, such as phenol, formaldehyde, mercuric chlorid, and copper sulphate, will destroy the virus in both cases, the virus of tobacco mosaic apparently being slightly more resistant in this

regard. The juices of both mosaic tobacco and cucumber plants may be diluted to 1 : 1,000 without affecting the power of infection, and dilutions of 1 : 10,000 will also produce the disease, giving, however, a lower percentage of infected plants.

The tobacco virus will pass through both the normal Berkefeld filters and those of the Chamberland type, while the cucumber virus passes only through the former. This difference is probably of minor importance, however, as Allard (4) has shown that the juices of tobacco mosaic are also rendered noninfectious if passed through a porous clay atmometer or through a $\frac{3}{4}$-inch layer of powdered talc, so that the behavior of the virus of both diseases is essentially the same as regards filtration.

POSSIBLE NATURE OF THE CAUSAL FACTOR.

Since no visible causal organism has been associated with the mosaic diseases, various theories have been advanced as to their nature and origin.

Woods (32), Koning (21), and more recently Freiberg (13) and Chapman (7) have held that enzyms, particularly oxidases, peroxidases, and catalase, are in some way connected with the cause of tobacco mosaic. Allard (4), on the other hand, has claimed that the disease is due to a specific pathogenic agent, probably an ultramicroscopic organism.

Both theories are based principally on work with the mosaic of tobacco, but they apply equally well to the corresponding disease of cucumbers. The evidence so far accumulated, however, seems to accord better with the theory advanced by Allard than with the enzymic hypothesis.

The virus of cucumber mosaic, like that of tobacco, seems to possess many of the characteristics of living matter. It loses its power of infection if heated above 70° C., is easily destroyed by chemicals, and will not withstand desiccation. In support of the enzymic theory of the nature of tobacco mosaic it is claimed that similar properties are possessed by enzyms, and this is undoubtedly true to a great extent. The virus of cucumber mosaic, however, does not have such marked enzymic qualities. Unlike that of tobacco, it loses the power of infection within 24 to 48 hours after the juices are expressed from the plant, regardless of the use of preservatives or the temperature at which it is kept, and will not withstand desiccation. It is quite conceivable, however, that an organism might be destroyed rapidly after removal from its natural environment in the plant tissues, especially as the juices of the cucumber undergo rapid chemical changes when expressed.

Another point which seems to support the theory of an organism as the cause of mosaic is the ability of the juices of mosaic plants to produce infection in a dilution of 1 : 10,000. Two or three drops

of such a dilution when pricked into the leaf of a healthy plant will produce the disease as rapidly and with as intense symptoms as when the undiluted juices are used. We have evidence here that the virus possesses the power of rapid increase, since all parts of the leaves and stem of the inoculated plant contain the virus within three to four days after inoculation. If we adopt the theory of an ultramicroscopic organism as the causal factor, this increase is readily explained, but examples of similar increase or multiplication are not commonly associated with enzyms.

The behavior of the virus in filtration may be used to support the theory, although the fact that the finer filters hold back the virus indicates that the particles composing the virus are probably colloidal in nature and of relatively large size. These qualities, however, neither exclude it from the class of enzyms nor from that of ultramicroscopic organisms.

In attempting to prove either of the above hypotheses it must be recognized that a great part of the available data is equally applicable to either theory and that no definite conclusion is as yet possible. It is the writer's opinion, however, that the causal agent possesses characteristics which tend to place it as an ultramicroscopic organism rather than as an enzym, but no property has yet been discovered which may not be characteristic of either agency.

On the other hand, the examples of diseases attributed to a so-called "filterable virus" or "ultramicroscopic organism" are numerous in animal pathology and are generally accepted, while as yet there are no demonstrated examples of diseases due to enzymic causes alone. In the case of tobacco mosaic the recent work of Allard (4) has nearly eliminated the oxidases from consideration, so that we at present can hardly attribute the disease to a definite type of enzym.

INFECTIOUS NATURE OF THE DISEASE.

The epiphytotic character of cucumber mosaic and its rapid spread to plants adjoining those first infected at once indicated that it was of an infectious nature, as proved by Jagger (17) and the writer (11). The first inoculation experiments by the writer were begun at Hamilton, Mich., in 1914, but as the plants used had no protection from insects and mosaic was already present in the field the results, although positive, did not permit definite conclusions. In 1915 the work was continued at Big Rapids, Mich. This district was practically free from the disease and the experimental fields had never before grown cucumbers. The possibility of outside infection, particularly from insects, was practically eliminated by using cages covered with cheesecloth to protect all plants in the inoculation experiments.

As the cages in most cases had no openings, they were lifted when inoculating the plants, but by exercising proper care and using the

cage as a partial shield during inoculation insects gained entrance very rarely. It was necessary to inspect all cages frequently to cut the tendrils which penetrated the cloth and to cover any small openings with heavy paint. All cages were set out as early in the season as possible, usually immediately after planting or by the time two to three leaves had appeared on the young plants. When plants were caged after the disease appeared in the field they were left at least 10 days before being used in inoculation experiments, in order that any previous accidental mosaic infection might have time to develop before inoculations were made.

Most of the cages used were of the same general type, the frame being of laths, with the exception of the corner uprights which were made of pieces from 1 to 2 inches square. In the earlier cages extra strips of lath were placed around the lower edge of the frame to allow it to be sunk 2 to 3 inches in the soil, but it was later found advisable to make the lower edges of light 4-inch boards. The cheesecloth used contained 24 to 30 threads per inch, the lower edge of the cloth being wrapped around strips of lath, which were then nailed to the bottom of the frame. Cages were also constructed with an opening in the top, but as they were difficult to build and often failed to remain insect proof, they were later abandoned, except for special purposes. Cages were used in practically all field work and reduced the factor of outside infection, which occurred only in rare cases, to a minimum. The caged plants grew luxuriantly and were only slightly affected by the shading of the cheesecloth.

INOCULATION EXPERIMENTS.

Inoculation of healthy vines with the juices of mosaic plants has proved that the causal agent of the disease is present in the tissues of the stems, leaves, and fruits of mosaic vines. A light wounding of the plant inoculated is apparently essential for infection, as the virus does not seem to penetrate the unbroken epidermis. The two methods of inoculation used differ chiefly in the form of the inoculum, the expressed juice of mosaic plants or their crushed tissues being used. Results by either method have been successful, as is shown below. Control inoculations were made in all cases, using caged plants and inoculating them with the expressed juices or crushed tissues of healthy plants (Pl. IX, *A*).

TESTS WITH EXPRESSED JUICES OF MOSAIC PLANTS.

In the earlier work the expressed juices of mosaic plants were used for inoculation. Portions of a mosaic plant, either leaves, stems, or fruits, were cut finely by means of a sterilized meat chopper, the juice pressed through cheesecloth, and in some cases filtered through filter paper, and the expressed juice used as the inoculum. The

inoculations were made in the stems, leaves, and in some cases in the fruits. In the earlier experiments a sterile hypodermic needle was used for inoculation and the inoculum injected rather deeply into the stem. It was found, however, that inoculations made by cutting off a leaf close to the stem and then pricking the inoculum into the wounded surface gave a higher percentage of infection. In leaf inoculations a drop of the inoculum was pricked into the leaf at various points with a sterile needle. The results of inoculations by these methods are shown in Table VIII.

TABLE VIII.—*Results of inoculations with the expressed juices of mosaic cucumber plants.*

Date inoculated.	Treatment.	Inoculum, expressed juice of—	Point of inoculation.	Number of plants inoculated.	Results.	
					Number of mosaic plants.	Date last observed
Aug. 2, 1915	Inoculated	Mosaic plant	Base and tip of stem.	4	3	Aug. 17, 1915
Do	Control	Healthy plant	do	3	0	Aug. 20, 1915
Aug. 4, 1915	Inoculated	Mosaic fruit	Young leaf and base of stem.	5	3	Aug. 14, 1915
Do	Control	Healthy fruit	do	3	0	Do.
Aug. 21, 1915	Inoculated	Mosaic fruit	Base of stem	8	8	Sept. 2, 1915
Do	Control	Healthy fruit	do	5	0	Do.
Nov. 19, 1915	Inoculated	Mosaic leaves	Young leaf	8	5	Nov. 26, 1915
Do	Control	Healthy leaves	do	6	0	Do.
Nov. 28, 1915	Inoculated	Mosaic plant	do	11	5	Dec. 6, 1916
Apr 8, 1916	do	do	Base of stem	7	6	Apr. 20, 1916
Do	Control	Healthy plant	do	8	0	Do.
Sept. 6, 1916	Inoculated	Mosaic plant	do	7	5	Sept. 15, 1916
Do	Control	Healthy plant	do	6	0	Do.

TESTS WITH CRUSHED TISSUES OF MOSAIC PLANTS.

In later work, experience proved that the easily prepared crushed tissues of mosaic plants formed a more constantly virulent inoculum than the expressed juices. Infected portions of the plant, either fruit, stem, or leaves, were crushed in a sterile dish after they had been cut into small fragments with sterile scissors or a scalpel. This material was inserted in a small cut in the stem made with a sterile scalpel. Such wounds were usually made in the lower portion of the stem, in some cases directly below the oldest leaf, but more commonly a leaf was cut off close to the stem and the incision made in the cut surface. A longitudinal cut, 3 to 5 millimeters long and 2 to 3 millimeters deep, was found sufficient, the small piece of crushed material being inserted in the wound, which healed rapidly. In other cases a small incision was made at the tip of the stem directly below a young leaf and a fragment of the crushed tissue inserted at this point. Inoculations with crushed material at either of these points, or both, ordinarily gave a slightly greater percentage of infection than was obtained by the injection of the expressed juices. It was found also that inoculations at two or three points were more generally successful than where a single inoculation was made.

This method is preferred chiefly because the preparation of the expressed juice requires time, and such extracts, unlike those from mosaic tobacco plants, lose their pathogenicity rapidly, usually having to be prepared fresh every day to insure success. In the case of the second method, fresh material is constantly available and its preparation takes little time.

Table IX gives results which are typical of inoculations by this method, but represent only a small fraction of the inoculations actually made.

TABLE IX.—*Results of inoculations with the crushed tissues of mosaic cucumber plants.*

Date inoculated.	Treatment.	Inoculum.	Point of inoculation.	Number of plants inoculated.	Results.	
					Number of mosaic plants.	Date last observed.
Aug. 24, 1915	Inoculated	Mosaic stem	Base of stem	3	3	Sept. 2, 1915
Do	Control	Healthy stem	do	3	0	Do.
Jan. 22, 1916	Inoculated	Mosaic leaf	do	4	4	Jan. 31, 1916
Nov. 3, 1916	do	do	do	6	4	Nov. 10, 1916
Do	Control	Healthy leaf	do	6	0	Do.
Feb. 17, 1917	Inoculated	Mosaic leaf	Tip of stem	5	3	Feb. 28, 1917
Do	Control	Healthy leaf	do	5	0	Do.
Mar. 11, 1917	Inoculated	Mosaic leaf	Base and tip of stem	12	8	Mar. 19, 1917
Do	Control	Healthy leaf	do	10	0	Do.
Mar. 15, 1917	Inoculated	Mosaic stem	Base of stem	12	7	Mar. 22, 1917
Do	do	Mosaic fruit	do	6	2	Do.
Do	Control	Healthy fruit	do	6	0	Do.
Mar. 29, 1917	Inoculated	Mosaic leaf	Base and tip of stem	7	5	Apr. 9, 1917
Do	Control	Healthy leaf	do	7	0	Do.
May 19, 1917	Inoculated	Mosaic leaf	do	4	4	May 30, 1917
Do	Control	Healthy leaf	do	4	0	Do.
June 20, 1917	Inoculated	Mosaic leaf	Tip of stem	8	5	July 4, 1917
Do	Control	Healthy leaf	do	8	0	Do.

RELATION OF INFECTION TO POINT OF INOCULATION.

STEM INOCULATIONS.

As shown by the preceding data, stem inoculations are successful in most cases, regardless of the point of inoculation. Inoculations made in wounds produced by the removal of a leaf are ordinarily more effective than at other points, possibly because the virus comes more directly in contact with the vascular bundles at such points.

LEAF INOCULATIONS.

Inoculation of any green leaf will produce mosaic infection, but the inoculation of young leaves gives a much higher percentage of mosaic plants than the inoculation of leaves that have reached their full development. The point of inoculation in the leaf, however, seems to make little difference.

An interesting fact, already proved by Allard (5) for tobacco mosaic, is that infection can take place through the trichomes. He has shown that infection may occur if the trichomes of healthy plants are cut or pinched with instruments which have previously been dipped in the juices of a mosaic plant or when the mosaic juices are painted on the trichomes. Similar experiments have been made with the cucum-

CUCUMBER EXPERIMENTAL FIELD AND SOIL OVERWINTERING TEST.

A, Experimental field at Big Rapids, Mich., in 1916, showing the various types of insect-proof cages used in making inoculations. Photographed by W. W. Gilbert. *B*, Soil overwintering test at Madison, Wis., September 19, 1917. This land grew a mosaic crop in 1916. The few large healthy plants were grown under cages. All the uncaged plants became diseased early and were dead or severely stunted. An insect hibernation cage is seen at the left.

MOSAIC AND HEALTHY CUCUMBER PLANTS FROM COMMERCIAL SEED.

A, Mosaic plant grown from commercial seed in a greenhouse at Madison, Wis., in 1917. Note the dwarfing as compared with *B*, the mottled and curled leaves, the shriveled cotyledons, and the dying of the edges of the first leaf. *B*, A healthy plant grown from the same seed as *A* and planted on the same date.

ber, the trichomes of mosaic and healthy leaves being brought in contact in such a way as to merely break the trichomes without injuring the epidermis of the leaf. Infection has resulted in most of the plants thus inoculated, as is shown in Table X.

TABLE X.—*Infection as a result of contact of trichomes of leaves of healthy and mosaic cucumber plants.*

Date inoculated.	Treatment.	Number of plants inoculated.	Results.	
			Number of mosaic plants.	Date last observed.
Apr. 20, 1916	Mosaic and healthy leaves in contact	2	2	Apr. 28, 1916
Do	Healthy leaves in contact (control)	2	0	Do.
Aug. 5, 1916	Mosaic and healthy leaves in contact	6	4	Aug. 16, 1916
Do	Healthy leaves in contact (control)	4	0	Do.
Feb. 21, 1917	Mosaic and healthy leaves in contact	12	9	Mar. 2, 1917
Do	Healthy leaves in contact (control)	12	0	Do.
Mar. 22, 1917	Mosaic and healthy leaves in contact	10	5	Apr. 4, 1917
Do	Healthy leaves in contact (control)	8	0	Do.
June 20, 1917	Mosaic and healthy leaves in contact	4	2	June 28, 1917
Do	Healthy leaves in contact (control)	4	0	Do.
Oct. 24, 1917	Mosaic and healthy leaves in contact	12	3	Nov. 2, 1917
Do	Healthy leaves in contact (control)	6	0	Do.

BLOSSOM INOCULATIONS.

No evidence has been obtained thus far which indicates infection through the blossoms. Numerous inoculations of male and female flower parts were made by pricking the expressed juice of a mosaic cucumber plant into the petals, stigmas, or anthers of the blossoms of healthy cucumber plants. From 3 to 8 blossoms on each of 38 plants have been inoculated in this way, but no infection has ever occurred. It is still a question, however, whether such infection may not occur, especially as the virus is known to be present in the blossoms of mosaic plants, as is shown on page 35.

FRUIT INOCULATIONS.

Inoculations into small fruits have given infection in a number of plants, as is shown in Table XI, but inoculations of fruits approaching maturity were nearly always unsuccessful.

TABLE XI.—*Results of inoculation of fruits of healthy cucumber plants.*

Date inoculated.	Treatment.	Inoculum.	Approximate age of fruits inoculated.	Number of fruits inoculated per plant.	Number of plants inoculated.	Results.	
						Number of mosaic plants.	Date last observed.
Mar. 20, 1916	Inoculated	Expressed juice of mosaic plant.	10 days	2	6	3	Apr. 5, 1916
Do	Control	Expressed juice of healthy plant.	do	2	6	0	Do.
Aug. 20, 1917	Inoculated	Expressed juice of mosaic plant.	5 days	2	8	3	Sept. 5, 1917
Do	do	do	21 days	2		3	Do.
Do	do	do	5 weeks	2	8	0	Do.
Do	do	do	do	1	3	1	Do.
Do	Control	Expressed juice of healthy plant.	10 days	2	7 10	0	Do.
Apr. 12, 1918	Inoculated	Expressed juice of mosaic plant.	7 days	2	5	3	May 3, 1918
Do	do	do	21 days	1		1	Do.
Do	Control	Expressed juice of healthy plant.	7 days	2	4 8	0	Do.

ROOT INOCULATIONS.

Attempts to produce mosaic by inoculations through the roots have so far given negative results. Seventy-four plants have been inoculated, using the following three methods:

(1) Inoculation of the roots was made with the expressed juice of a mosaic plant or by the insertion of crushed fragments of mosaic leaves in wounds in the larger roots at a distance of 1 to 6 inches from the stem.

(2) Mosaic vines were passed through a food chopper and the ground material mixed with sterilized soil. Healthy plants were then transplanted in the soil thus prepared, the process of transplanting insuring root injuries as points of possible infection.

(3) Roots of healthy plants were injured at points close to the surface of the soil, and the expressed juice of mosaic plants was then poured about the stem close to the point of injury.

No infection has resulted from any of these methods, and the disease apparently is not transmitted through the roots.

RELATION OF AGE AND VIGOR OF PLANT GROWTH TO SUSCEPTIBILITY.

The percentage of successful inoculations on the cucumber and other curcurbits seems to be directly related to the age of the plant and the vigor of its growth. Inoculations on seedling plants, even up to the time that three or four leaves have appeared, give a low percentage of infection, and it is especially difficult to secure infection on seedlings during the development of the first true leaf.

A little later, however, when the plants begin to grow rapidly and have developed six to eight leaves, most of those inoculated develop the disease. This period of susceptibility then continues until the plant becomes old and growth has nearly ceased, at which stage the percentage of successful inoculations again becomes low. Plants growing under unfavorable conditions which cause a stunting or retardation of growth are also less susceptible to the disease and show the first symptoms more slowly than those which are growing rapidly.

These facts indicate that the age and vigor of the plant have a direct relation to its susceptibility to mosaic. While this is probably true, it is a question what relation these factors bear to the susceptibility of the plant and its response to the disease stimulus. The rapidly dividing cells of the tissues still in the process of development apparently react to the disease stimulus more readily than the older tissues. It is possible that these younger tissues offer conditions more favorable to the multiplication of the virus [1] and consequent infection than the older cells of mature plants.

It has been suggested that older plants may contain the virus but show the symptoms in so mild a form that they can not be detected. However, this has apparently been disproved by making inoculations from old plants which had been previously inoculated with

[1] The term virus is used here in the commonly accepted sense of the infective agent of a disease with which no visible organism has been associated.

mosaic but showed no symptoms of the disease. No such plant has ever shown evidence of containing the virus, as all inoculations from them gave negative results, and it is probable that a definite difference in susceptibility occurs, depending on the age of the plant.

LOCATION OF THE VIRUS IN THE PLANT.

The virus of curcubit mosaic is distributed throughout practically all parts of the plant, with the possible exception of the roots. The data given in Tables VIII to X show that the juices of stem, leaves, and fruits of mosaic cucumber plants are all capable of producing infection. All the living leaves of a mosaic plant contain the virus, regardless of age or the presence of actual mosaic symptoms, its thorough distribution in the leaf cells being shown by the results of inoculation by contact of healthy and mosaic trichomes.

VIRUS IN THE FLOWER PARTS.

The disease can also be produced by inoculation with the flower parts of diseased plants. Inoculations were made with petals, anthers, and stigmas of mosaic cucumber plants by carefully dissecting out with sterile instruments the portion of the flower desired without breaking other parts and crushing the material in a sterile dish with a few drops of sterile water. This crushed mass was then used for stem inoculations, the results being positive in each case, as shown in Table XII. Allard (3) has shown that the virus of tobacco mosaic is also present in the anthers, filaments, and even in the placental column and integument of the ovule of infected plants.

TABLE XII.—*Results of inoculations with the crushed tissues of flower parts of mosaic cucumber plants.*

Date inoculated.	Treatment.	Inoculum.	Number of plants inoculated.	Results.	
				Number of mosaic plants.	Date observed.
Sept. 7, 1916	Inoculated	Corolla of mosaic blossom	3	3	Sept. 23, 1916
Do	do	Stigma of mosaic blossom	3	3	Do.
Do	do	Anthers of mosaic blossom	3	2	Do.
Mar. 12, 1917	do	do	10	3	Mar. 21, 1917
Do	Control	Anthers of healthy blossom	8	0	Do.
Do	Inoculated	Stigma of mosaic blossom	8	5	Do.
Do	Control	Stigma of healthy blossom	8	0	Do.
Do	Inoculated	Corolla of mosaic blossom	10	6	Do.
Do	Control	Corolla of healthy blossom	8	0	Do.

VIRUS IN THE FRUITS.

Inoculations made with the crushed tissues of mosaic cucumber fruits have shown that the virus is apparently present in fruits of all ages. The inoculum was prepared by removing small portions of the tissues close to the ovule with a sterile scalpel and crushing them in a sterile dish, the inoculations being made in the stems of

healthy cucumber plants by the method described on pages 30 to 32. The fruits used as inocula were of various ages, including young fruits about 1½ inches in length, large, partially mature fruits that were beginning to turn yellow, and yellow and matured fruits such as are collected for seed. The very young and the partially mature fruits showed the presence of the virus in all cases (Table XIII), and while the inoculations from the mature fruits were not so constantly successful they produced the disease in a number of plants. The presence of the virus in the ovule itself has not been demonstrated, as it is difficult to remove the ovules from a watery fleshy fruit like the cucumber without carrying traces of the juices of other parts of the fruit.

TABLE XIII.—*Results of inoculations with crushed tissues of mosaic cucumber fruits of various ages.*

Date inoculated.	Treatment.	Inoculum, crushed tissues of—	Point of inoculation.	Number of plants inoculated.	Results.	
					Number of mosaic plants.	Date last observed.
Sept. 10, 1917	Inoculated	Young mosaic fruit	Base and tip of stem.	5	3	Sept. 21, 1917
Do	do	Partly mature mosaic fruit.	do	6	4	Do.
Do	do	Mature, yellow mosaic fruit.	do	5	0	Do.
Do	Control	Healthy fruit	do	8	0	Do.
Sept. 20, 1917	Inoculated	Young mosaic fruit	do	10	6	Oct. 14, 1917
Do	do	Partly mature mosaic fruit.	do	10	3	Do.
Do	do	Mature, yellow mosaic fruit.	do	10	4	Do.
Do	Control	Healthy fruit	do	10	0	Do.

VIRUS IN THE MATURE SEED.

Although the young fruits and even fruits at maturity may have the virus present, as is shown in Table XIII, it has never been detected in any portions of the seed which have been removed from the fruit and subsequently dried. The drying process probably accounts for its absence from the seed coat, since the juice of mosaic cucumber plants soon loses its infectious quality if subjected to desiccation. Tests have been made of both seed coat and embryo in 37 inoculations, the material being crushed in sterile distilled water and both the water extract and the crushed tissue used for inoculation, but no infection has ever occurred. Allard (3) has found that the virus of tobacco mosaic may be present in the mature dry seeds of mosaic tobacco plants, and this is consistent with the fact that the virus of tobacco mosaic is more resistant to drying than that of cucurbit mosaic. At the present time, however, there is rather definite proof that the mosaic disease of cucumber may be carried over in the seed. (Pl. I, *A*.) If such is the case, it is possible that the virus is occasionally present in the seed, although perhaps so rarely that a large number of inoculations would be necessary to demonstrate its presence.

VIRUS IN THE ROOTS.

Inoculations from the roots of mosaic plants have so far given negative results, and it is doubtful whether the virus is present in these parts as in the remainder of the plant. Allard (1) notes that the roots of mosaic tobacco plants contain the virus in many cases. In the case of cucumber mosaic, inoculations have been made from the roots of plants of various ages, using either the expressed juice of the root tissues or crushed fragments of the tissues themselves. Inoculations have been made into the stems, leaves, or roots of 58 plants, but no infection has occurred.

VIRUS DISTRIBUTION IN THE PLANT.

RAPIDITY OF SPREAD OF THE VIRUS.

In all inoculations with cucurbit mosaic the earliest signs of infection appear in the youngest leaves. This is also the case with tobacco mosaic and other diseases of this type, the leaves which are in process of development seeming to respond most rapidly to the disease stimulus. The time required for the development of these symptoms is usually the same, regardless of the point of inoculation, indicating that the virus is rapidly distributed to all parts of the plant. The youngest leaves of the cucumber develop mosaic symptoms as rapidly when inoculation is made at the base of the stem as when made in the leaf itself. This does not prove that the virus is immediately distributed throughout the plant, however, for the infective principle is present in the cells for some time before visible symptoms occur and it is probable that the virus is present in greater amount near the point of inoculation for at least a short time after inoculation. The older leaves, however, do not show definite signs of the disease for some time, and no external indication of the disease appears until the young leaves develop mosaic symptoms. The accumulation of the virus at the point of inoculation earlier than at the growing tip has been demonstrated in large cucumber plants where inoculation was made at the base of the stem about 3 inches below a small lateral shoot which was 6 inches in length. The leaves of such a shoot showed the symptoms of the disease 12 hours before it appeared in the young leaves at the tip of the main runner, which were 30 inches beyond the point of inoculation. This occurs occasionally in large vines, but in most cases there is no appreciable difference in time between the development of symptoms at various points, and the distribution of the virus through the plant must be comparatively rapid.

Experiments have shown that the juice of an inoculated plant may be infectious 18 to 48 hours previous to the appearance of any definite mosaic symptoms and also, as already indicated, that the juice from leaves near the point of inoculation may become infec-

tious 12 hours earlier than that from leaves at more distant points. In these tests cucumber plants were inoculated either at the base or tip of the stem, and other plants were then successively inoculated with fragments of leaf tissue from various points on the plant first inoculated. These later inoculations were made every 12 or 24 hours. The results of this work are given in Table XIV. The fact that mosaic diseased plants possess the power of infection before the appearance of visible symptoms is a most important factor in its relation to disease dissemination and control. Efforts to control cucumber mosaic in the field by the eradication of all diseased plants as soon as they appeared have met with little success, largely because of the fact that a great number of plants were constantly serving as a source of infection during the period when their juices were infectious, but before visible symptoms had appeared.

TABLE XIV.—*Occurrence of mosaic virus in inoculated cucumber plants prior to the appearance of visible symptoms.*

Plant No.	Date of first inoculation.	Point of inoculation.	Date first symptoms were noted.	Part used for secondary inoculations.	Date of first successful secondary inoculation.	Time virus was present before first symptoms appeared (hours).
1	July 18, 1916	Tip of stem...	July 22, 1916	Leaf at tip of stem.....	July 22, 1916	0
2	.....do.....	Base of stem..	July 24, 1916	.....do.....	July 23, 1916	24
3	.....do.....	Tip of stem...	.....do.....	.....do.....	.....do.....	18
4	.....do.....	Base of stem..	.....do.....	Leaf at base of stem.....	.....do.....	24
			.....do.....	Leaf at tip of stem.....	.....do.....	24
5	.....do.....	Tip of stem...	July 25, 1916	Leaf at base of stem.....	July 24, 1916	24
			.....do.....	Leaf at tip of stem.....	July 23, 1916	36
6	Aug. 8, 1917	Base of stem..	Aug. 15, 1917	Tip leaf of shoot 6 inches from point of inoculation.	Aug. 13, 1917, 6.45 a. m.	38
			Aug. 16, 1917	Tip leaf of main stem 30 inches from point of inoculation.	Aug. 13, 1917, 4 p. m.	48

METHOD OF DISTRIBUTION OF THE VIRUS.

The distribution of the virus throughout the plant is probably effected either by diffusion through the parenchymatous cells or by conduction through the vascular elements. While the question is difficult to determine definitely, it seems more probable, in the writer's estimation, that the vascular system is most important, although diffusion may also be a factor. When inoculation is made at the base of the stem, the first symptoms, which invariably appear in the young leaves, develop as rapidly as when inoculation is made directly into these leaves. It thus appears that the distribution is rapid, since the young leaf tissue may contain the virus within three days after inoculation in sufficient amount to produce infection in other plants. It would seem that diffusion alone would be too slow to allow the virus to reach all parts of the plant in as short a time as

it does when inoculation is made in the stem tissues. The fact that the pores of a Chamberland filter can hold back the particles of infectious material also leads to a belief that such particles may be colloidal in nature and not likely to pass readily through the cell membranes.

Allard (5) has shown in the inoculation of older leaves of tobacco plants that severing the lateral veins from the midrib or cutting the base of the midrib itself does not appreciably increase the time usually required for the virus to reach the stem and pass to the young leaves. He believes that in such cases "multiplication and diffusion of the virus from cell to cell, aided perhaps by the fine anastomosing lateral veins, would sooner or later allow the virus to reach the petiole and pass to the rest of the plant." The writer is inclined to believe from the results of work with cucumber aphids that conduction through the fine veins is a more important means of transference than diffusion. These insects apparently attack only the small veins of the leaf, as shown both by observation and by stained microtome sections of aphids killed and embedded while still attached to the leaf. In all such sections the sucking apparatus of the insect extended into the small leaf veins. Further support of this fact is given by Woods (31) in his work on stigmonose of carnation, in which he shows drawings and photomicrographs which indicate that the aphis attacks the vascular elements. Since aphids are the most consistently successful agency of inoculation for cucurbit mosaic yet discovered, it seems likely that their high percentage of infection is due to the introduction of the virus at a point where it is most rapidly carried to all parts of the plant. Stem inoculations made by cutting off a petiole and pricking the juices of mosaic cucumber plants into the region about the bundles of the leaf trace give about double the percentage of infection that occurs when the virus is pricked into the stem at random, indicating again that the vascular system is concerned.

The portion of the vascular system especially concerned is still rather indefinite, but the work with aphids again furnishes a clue. Woods's work before mentioned (31) indicated that the soft bast parenchyma cells of the phloem were the ones particularly attacked. Other observers have also recorded the same facts, and the writer's work along this line, although not extensive, indicates that the cucumber aphis punctures the cells of the phloem. If this is true it may be possible that the phloem is at least as important as the xylem in the distribution of the virus, since the inoculation work with aphids is so universally successful. Beijerinck (6) is of the opinion that the phloem is the means of distribution of tobacco mosaic, since inoculation of the older leaves first produces symptoms in the young leaves, and he therefore concludes that the virus is carried in the descending sap flow.

All the above conclusions are merely tentative, however, particularly in regard to the rôle of the phloem in the distribution of the virus. The vascular elements, however, seem to offer the most probable channel for the rapid distribution of infectious material through the plant.

INCUBATION PERIOD.

The incubation period of cucurbit mosaic varies somewhat with the age of the plant inoculated. In young cucumber plants which are growing rapidly the first visible signs of the disease may appear within 4 to 5 days following inoculation, and the incubation period in such plants will rarely exceed 8 or 9 days. In the case of older plants which are growing less rapidly, the period of incubation is often longer and the first symptoms may not develop for 12 or 14 days, although these plants generally do not require over 10 days for the appearance of the symptoms.

The incubation period for plants of the same age and vigor of growth is quite constant, and where several plants are inoculated at the same time those which are infected show the signs of the disease within 3 to 4 days after the earliest symptoms appear. Plants which do not develop mosaic symptoms within this period usually remain healthy.

The incubation period of the other cucurbits is very nearly the same as that of the cucumber and varies within approximately the same limits. In the case of large squash and gourd plants the incubation period is slightly longer, ordinarily, than that of most other cucurbits, and usually extends over 12 or 15 days. On the younger plants of all species, the first symptoms will normally develop within 7 to 9 days after inoculation. The general incubation period may be said to lie between 4 and 15 days, with the average period varying from 7 to 12 days for all species.

MOSAIC TRANSMISSION.

Many mosaic diseases are of such a nature that field operations, in which healthy and diseased plants are handled in succession, tend to further disseminate the disease. This has been demonstrated by Hunger (15) for tobacco mosaic and is particularly true with the mosaic on cucumber, the method of growing and harvesting the crop being such as to favor the spread of infection throughout the season. As infection may occur whenever the juices of a mosaic plant come in contact with slight wounds in healthy vines, any thinning, training, or other handling of mosaic and healthy plants may produce infection. That mere handling of the plants may result in disease transmission has been proved by bruising the leaf of a mosaic vine between the fingers and then handling the leaves of healthy plants in a simi-

lar manner, but without crushing them. The results, shown in Table XV, indicate the ease with which infection may be brought about by this method.

TABLE XV.—*Infection resulting from successive handling of mosaic and healthy cucumber plants.*

Date.	Treatment.	Number of plants.	Results.	
			Number of mosaic plants.	Date observed.
Aug. 24, 1916	Leaves of diseased and healthy plants handled in succession.	5	3	Sept. 4, 1916
Do.....	Leaves of two healthy plants handled in succession (control).	4	0	Do.
Aug. 11, 1917	Leaves of diseased and healthy plants handled in succession.	6	4	Aug. 30, 1917
Do.....	Leaves of two healthy plants handled in succession (control).	6	0	Do.
Mar. 12, 1918	Leaves of diseased and healthy plants handled in succession.	10	6	Mar. 28, 1918
Do.....	Leaves of two healthy plants handled in succession (control).	8	0	Do.

TRANSMISSION DUE TO CULTURAL OPERATIONS.

TRAINING AND REMOVAL OF INTERTWINED PLANTS.

The evidence reviewed above shows that handling may spread infection at any time, but it is more common later in the season when the plants have made a heavy growth. As the vines lengthen it is necessary constantly to train them back from the center of the row, and in picking they are also lifted and pulled about. These operations produce many small abrasions, as the adjacent vines are usually interlaced, and when a mosaic and healthy plant occur together these slight wounds very often result in infection. It is commonly observed where few insects are present that the disease spreads along the row to the plants adjacent to the first isolated cases of infection. That this is due to such means as those mentioned above was strikingly demonstrated at Big Rapids, Mich., in 1916. The mosaic appeared at several points in a small field, and during the remainder of the season, in an effort to control it, all plants showing the disease were removed as soon as the first definite symptoms were observed. It was noted that, 10 days after the removal of the diseased individuals, plants in the same row and immediately adjacent to them began to show symptoms of mosaic, the disease progressing down the rows in both directions from the original diseased plants. In this experiment the vines were removed carefully, with the idea of avoiding infection, but slight injuries were unavoidable, and it is evident that such infection is constantly occurring throughout the season. It is probable that the factor of trichome infection is of special importance in cases of this sort, since the injuries need be but slight to produce infection through this channel.

INFECTION BY WALKING ON PLANTS.

In many cases the vines grow so thickly that a certain amount of injury from treading on the plants during picking is unavoidable, particularly where the rows are narrow. This probably has produced infection in many cases, one of which was noted by Mr. W. W. Gilbert in 1915 at Muscatine, Iowa. A party of several persons had crossed a field of seed cucumbers where the growth was rank and many mosaic plants were present. In so doing, vines were necessarily crushed under foot. A field of healthy plants in the neighborhood was visited soon after. The growth was heavy in this second field also, and further trampling of the plants occurred. Three weeks later the path taken through the second field was distinctly marked by mosaic infection, while the remainder of the field was still free from mosaic. This doubtless was an extreme case, but it is likely that some infection takes place in this way in many fields.

INFECTION BY PICKING.

In harvesting pickling cucumbers, they are picked every day or every other day, in order to secure small fruits. The fruits are broken off by hand in rapid succession and the juices, which soon cover the fingers, furnish an ideal and rapid means of mosaic transmission throughout the season. This has been demonstrated, as shown in Table XVI, by alternately picking fruits from diseased and healthy vines. The fruits were merely broken off with the thumb nail and no effort was made to produce any greater wounding or adherence of juices to the fingers than is common in field operations.

TABLE XVI.—*Experiments to prove mosaic infection of cucumber plants due to picking.*

Date.	Treatment.	Number of fruits picked per plant.	Number of plants inoculated.	Results.	
				Number of mosaic plants.	Date last observed.
Aug. 5, 1916	Fruits picked from healthy plant after picking mosaic fruits.	2	4	3	Aug. 21, 1916
Do	Fruits picked from healthy plants only (control).	2	4	0	Do.
Oct. 25, 1916	Fruits picked from healthy plant after picking mosaic fruits.	2	5	3	Nov. 15, 1917
Do	Fruits picked from healthy plants only (control).	2	5	0	Do.
Nov. 24, 1917	Fruits picked from healthy plant after picking mosaic fruits.	1	7	4	Dec. 8, 1917
Do	Fruits picked from healthy plants only (control).	1	7	0	Do.

WOUNDS NECESSARY FOR CONTACT INFECTION.

In all the above work, the wound factor must be emphasized, since infection has never been known to occur where diseased and healthy vines were in undisturbed contact. Hundreds of cases have been observed where only a portion of the plants in a cage would

develop mosaic symptoms after inoculation, and these healthy and diseased plants would remain intertwined for several weeks without further cases of mosaic appearing, no insects being present to cause infection.

INSECT TRANSMISSION.

While dissemination of mosaic often occurs by the means already noted, a large part of the infection in the field is often due to insects attacking the cucumber.

TRANSMISSION BY APHIS GOSSYPII.

The importance of insects as a source of infection has been demonstrated by both Jagger (17) and Doolittle (11) in the case of the melon aphis (*Aphis gossypii* Glover), and field observations have repeatedly shown that this insect may be instrumental in the production of epidemics of cucumber mosaic. At Hamilton, Mich., in 1914, a severe attack of aphids occurred over a wide territory during the latter part of July, when mosaic was present in a number of fields but had not become widely distributed throughout the district. As the aphids spread from field to field and increased in numbers, it was noted that a severe outbreak of the disease usually occurred about ten days after the appearance of the insects. Within a month most of the fields about Hamilton showed from 60 to 90 per cent of mosaic infection, and the crop was practically destroyed. Similar results of aphid infestation were noted in Michigan in 1916 and in Wisconsin in 1917, but over less extended areas.

TABLE XVII.—*Transmission of cucumber mosaic by Aphis gossypii.*

Date.	Treatment.	Source of aphids.	Number of aphids placed on each plant.	Number of plants inoculated.	Results.	
					Number of mosaic plants.	Date observed.
Aug. 6, 1915	Inoculated	Mosaic cucumber leaves	25 to 30	8	8	Aug. 21, 1915
Aug. 23, 1915	do	do	15	8	8	Sept. 3, 1915
Do	Control	Healthy cucumber leaves	30	8	0	Do.
Aug. 24, 1915	Inoculated	Mosaic cucumber leaves	10	9	9	Do.
Do	Control	Healthy cucumber leaves	20	6	0	Do.
Aug. 30, 1915	Inoculated	Mosaic cucumber leaves	5	7	7	Sept. 10, 1915
Do	do	do	3	6	6	Do.
Do	Control	Healthy cucumber leaves	15 to 25	10	0	Do.
Aug. 4, 1916	Inoculated	Mosaic cucumber leaves	10	6	6	Aug. 14, 1916
Aug. 10, 1916	do	do	8	9	9	Aug. 18, 1916
Aug. 5, 1917	do	do	6	12	9	Aug. 16, 1917
Aug. 17, 1917	do	do	6	8	7	Aug. 29, 1917
Do	do	do	3	8	6	Do.
Do	do	do	2	8	2	Do.
Do	Control	Healthy cucumber leaves	8	8	0	Do.
Sept. 1, 1917	Inoculated	Mosaic cucumber leaves	5	6	5	Sept. 10, 1917
Do	do	do	3	6	1	Do.
Do	do	do	1	6	0	Do.
Do	Control	Healthy cucumber leaves	10	8	0	Do.

The agency of the insect in transmitting mosaic has been proved by experiments in which aphids taken from a mosaic cucumber plant were placed on healthy plants under cages. In nearly all cases,

this has resulted in the infection of all the plants on which the insects were placed (Pl. II, *B*). Aphids transferred from one healthy plant to another have never produced the disease.

The high percentage of mosaic infection obtained from inoculation by means of aphids from diseased plants (Table XVII) is probably due to the fact that, being a sucking insect, it introduces the virus into those tissues which will distribute it most rapidly throughout the plant. The number of aphids necessary to produce infection is small, infection having occurred where only three of the insects were placed on each plant, but where only one to three individuals are used the percentage of infection is often lowered.

TRANSMISSION BY DIABROTICA VITTATA.

Although the cucumber aphis may be responsible for severe epidemics of mosaic, the striped cucumber beetle (*Diabrotica vittata* Fabr.) is probably the most important insect agent in the transmission of the disease. While the aphis can cause extensive and rapid mosaic dissemination when present in large numbers, it appears only at intervals in most districts and is then often confined to a few fields. The striped beetle, however, is common to most cucumber-growing sections and usually occurs in considerable numbers throughout the season. It is a very active and voracious insect and attacks practically all parts of the plant, but more particularly the leaves. While aphids will nearly always produce infection when transferred from mosiac to healthy plants, the percentage of infection is much lower in the case of the beetle. Being a chewing insect, it transmits the disease only when mosaic plant juices are carried on its mouth parts to wounds in healthy plants and are then distributed throughout the tissues. Many of these insect wounds are slight, however, and are often located in the blossoms and at other points which are not favorable to the rapid distribution of the virus in the plant, so that it is probable that drying out often prevents infection. This low percentage of infection is offset by the fact that the beetles are numerous and active, constantly feeding first on one plant, then on another, with the result that the chances for infection are many. In localities where beetles have been numerous the disease has spread very rapidly and usually over a wide territory, as the insect often travels considerable distances.

Experimental work has given definite proof of the agency of beetles in mosaic transmission. Beetles captured directly from mosaic vines and placed on healthy plants under cages have produced the disease in numerous cases. Usually a considerable number of insects were placed in each cage, owing to the lower chances of infection before mentioned, but the numbers were no greater than were com-

monly found on an equal number of plants in the same field. Checks were not run with the insects, as in field experiments it was impossible to determine with certainty whether or not the beetles had previously fed on mosaic vines. The results of these tests, shown in Table XVIII, indicate that the beetle must be an important agent of transmission in most fields.

TABLE XVIII.—*Transmission of cucurbit mosaic by caging Diabrotica vittata over healthy cucumber plants, as shown by experiments made at Big Rapids, Mich., and Madison, Wis.*

Location and date beetles were caged.	Number of beetles per cage.	Source of the beetles.	Method of infection of beetles.	Number of plants per cage.	Total number of plants.	Results.	
						Number of mosaic plants.	Date last observed.
Big Rapids, Mich.:							
July 28, 1916	8	Madison, Wis	Natural infection	2	16	3	Aug. 12, 1916
Aug. 4, 1916	12	Big Rapids, Mich	Fed on mosaic plants in cage.	2	2	1	Aug. 17, 1916
Aug. 5, 1916	8	do	do	3	12	3	Aug. 21, 1916
Aug. 11, 1916	10	Madison, Wis	Natural infection	3	12	0	Sept. 5, 1916
Aug. 14, 1916	8	Big Rapids, Mich	Fed on mosaic plants in cage.	2	10	4	Do.
Madison, Wis.:							
Aug. 11, 1917	35	Madison, Wis	Natural infection	8	8	3	Aug. 21, 1917
Do	32	do	do	4	4	1	Do.
Do	28	do	do	7	7	3	Do.
Do	30	do	do	9	9	5	Do.
Aug. 23, 1917	30	do	do	7	7	3	Sept. 4, 1917
Do	30	do	do	5	5	1	Do.
Do	30	do	do	5	5	3	Do.
Do	30	do	do	6	6	2	Do.
Sept. 1, 1917	20	do	do	6	6	1	Sept. 12, 1917
Do	22	do	do	5	5	2	Do.
Do	27	do	do	6	6	1	Do.
Oct. 15, 1917	100	do	(Cucumber vines killed by frost.)	40	40	0	Oct. 30, 1917
Oct. 22, 1917	40	do	do	18	18	0	Nov. 17, 1917
Oct. 27, 1917	38	do	do	15	15	0	Nov. 21, 1917
Nov. 16, 1917	45	do	do	15	20	0	Do.

It will be noted that no beetles collected on or after October 15, 1917, produced infection. All cucumber plants were killed by frost about September 25, and all beetles collected in cucumber fields after that date had fed on other plants or on squash and cucumber fruits which still lay in the fields and had not fed on mosaic cucumber vines for three or more weeks prior to collection. In these tests plants were placed in a large cage in the greenhouse which held 40 plants in small pots, and the beetles were allowed to feed on the young cucumber plants for several days. The results indicate that this insect can transmit the disease for only a short time after feeding on mosaic plants.

DIABROTICA DUODECIMPUNCTATA AS A CARRIER OF THE DISEASE.

The 12-spotted cucumber beetle (*Diabrotica duodecimpunctata* Oliv.) is also an agent of transmission of cucurbit mosaic in the field. This insect is so similar to the striped cucumber beetle that

all statements regarding one insect will probably apply equally well to the other. The work with *Diabrotica duodecimpunctata* was done in the same manner as that with *D. vittata*, and the results were similar, as is shown in Table XIX.

TABLE XIX.—*Transmission of cucumber mosaic by Diabrotica duodecimpunctata in experiments at Madison, Wis.*

Date beetles were caged.	Number of beetles per cage.	Source of beetles.	Method of infection of beetles.	Number of plants per cage.	Results.	
					Number of mosaic plants.	Date of last observation.
Aug. 16, 1917	35	Madison, Wis.	Natural infection	4	3	Aug. 30, 1917
Do.	35	do.	do.	3	2	Do.
Do.	35	do.	do.	5	1	Do.
Aug. 21, 1917	25	do.	do.	5	3	Sept. 20, 1917
Do.	25	do.	do.	5	3	Do.
Do.	25	do.	do.	5	1	Do.
Do.	25	do.	do.	5	2	Do.
Do.	25	do.	do.	5	0	Do.

TRANSMISSION BY OTHER CUCUMBER INSECTS.

It is quite possible that other insects attacking the cucumber may also transmit the disease, but they are probably of less importance. The tarnished plant bug (*Lygus pratensis* L.), being a sucking insect, very probably carries the disease to some extent, but the few tests made with this insect have given negative results. Thrips (*Thrips tabaci* Lind.), the red spider (*Tetranychus telarius* L.), and the white fly (*Aleyrodes vaporariorum* Westw.) have shown no indications of being carriers of the disease, although greenhouse observations have included a large number of cases where the insects were constantly feeding on adjacent healthy and mosaic plants. This is in agreement with the work of Allard (5), who found that neither the white fly nor the red spider acted as carriers of tobacco mosaic in the greenhouse.

TRANSMISSION BY BEES.

The cucumber and other cucurbits which blossom heavily throughout the season are constantly frequented by great numbers of bees. These insects might therefore be important agents in disease dissemination, provided they carry the infection. However, the evidence thus far secured indicates that little, if any, infection is so carried. To test this possibility bees were taken directly from the blossoms of mosaic plants and then caged with healthy plants for some time. Infection has never been known to occur, although trials have been made with 53 plants. As stated on page 33, all attempts at artificial inoculation through the blossoms have so far failed, and it is likely that if infection occurs at this point it is

rather rare. Field observations, moreover, give little support to the theory that bees are a factor in mosaic dissemination, since in many cases where few other insects were present the spread of the disease would in all probability still have been rapid if the bee were a carrier, whereas in such cases the increase of infection was relatively slow.

POLLEN AS A CARRIER OF INFECTION.

The work with bees has also brought up the question of possible infection through the fertilization of flowers of healthy plants with pollen from the blossoms of mosaic plants. This has been tested by artificial fertilization of healthy cucumber flowers with pollen from mosaic vines, the operation being performed with a camel's-hair brush to avoid wounding the flower parts. Although many fruits were thus produced, no mosaic infection has ever been found. In these experiments care was taken to protect the blossoms from outside pollen and to make certain that the fruits set were pollinated only with pollen from mosaic plants.

METHOD OF OVERWINTERING.

FIELD OBSERVATIONS.

Since the factors concerned in the field transmission of the disease are sufficiently well established to account in large measure for its rapid dissemination, the problem of its overwintering and reappearance each year is perhaps most important, aside from the actual cause, in relation to possible methods of control.

As before stated, the origin of the disease seems unrelated to soil or climatic conditions, and its appearance is dependent on some specific infection. For the past four years it has appeared in Michigan, Indiana, and Wisconsin at approximately the same time, the first reports of its occurrence each year having been somewhere between July 7 and July 20, at a time when the plants were from 5 to 6 weeks old and had from 8 to 10 leaves. Infection of very young plants is rare and has usually appeared in cases where the seed was planted very late in the season, at a time when infection was already present on older plants in the same field.

The regularity in the appearance of the disease extends to most localities in the States mentioned, and a survey of the cucumber-growing districts of Michigan or Wisconsin about the middle of July usually shows the disease developing almost simultaneously in most localities where it was severe the year previous. In many cases the first infections are found on a few plants scattered through the field, or perhaps on plants in a single center, including six to eight vines within a short distance of one another, and from these it spreads gradually to neighboring plants. This manner of appearance and

subsequent development is most common, but in some instances the disease has suddenly developed in epiphytotic form, a striking example of which occurred at Madison, Wis., in 1916, followed by a second and more severe case in 1917. During 1916 the writer was not at Madison, but Dr. M. W. Gardner reported that the disease appeared early in July and in a short time in many fields had affected 25 to 50 per cent of the plants.

During 1917, frequent observations were made on the experimental fields at Madison from the beginning of the season. The fields under observation consisted of six plats within a radius of 1½ miles and varied in size from one-half to 1½ acres. These were practically the only large cucurbit fields in the locality, with the exception of a few plantings of muskmelon and squash. Up to July 19 no mosaic had appeared on any cultivated cucurbits in the locality so far as known. On this date, however, two of the six plats showed a number of cases of mosaic in its earliest stages. On July 21 definite symptoms of mosaic were noted on 25 per cent of the plants in these two plats and on a few plants in each of the remaining four plats. On July 26 at least 50 per cent of the plants in the first two plats and from 3 to 18 per cent of the plants in the other plats were affected. A survey of gardens in the vicinity also showed a few cases of the disease on July 26. The plants noted between July 19 and 26 were all in the early stages of the disease, and so far as could be determined all the infection must have occurred within seven or eight days. Such a sudden development of the disease would seem to indicate that some agent of dissemination was present which produced rapid and widespread infection at a definite period.

The most probable factors in overwintering would seem to be: (1) Soil containing refuse from mosaic vines of previous seasons; (2) the use of seed from diseased plants; (3) possible wild cucurbit hosts; (4) the presence of an identical disease on plants of another family; or (5) some insect agency. Most of the work has therefore been conducted with these possibilities in mind.

SOIL AS A SOURCE OF INFECTION.

It has been shown that the expressed juices of mosaic plants remain infectious for only a short period and that dried tissues of such plants have never shown the presence of the virus. This indicates that soil containing the remains of mosaic plants is not likely to prove a source of infection to further crops.

GREENHOUSE EXPERIMENTS.

During the winter of 1916, 40 plants were grown in the greenhouse in soil from a field at Hamilton, Mich., where mosaic had been serious for three successive years. The plants were transplanted to

insure root injury as a point of possible infection. As controls, 40 plants were transplanted into the same soil after it had been sterilized with steam at 15 pounds' pressure for three hours. Plants grown in this soil, whether sterilized or not, remained healthy in all cases.

During the month of August, 1916, a large number of mosaic cucumber vines were buried about 6 inches deep in sandy loam at Big Rapids, Mich., and left until the following year. In July, 1917, some of this soil containing the remains of the mosaic vines was taken to Madison, Wis., and used in the following experiments:

(1) Cucumbers were grown in the soil directly from seed, and small seedlings were also transplanted into pots of the same soil. In each case, 25 plants were used, together with 30 check plants grown in the same soil after steam sterilization for three hours at 20 pounds' pressure. All plants remained healthy.

(2) A 50-gram portion of the soil, containing a number of fragments of dried stems and leaves, was mixed with 50 c. c. of sterile distilled water and allowed to stand for 12 hours. This extract was then filtered and the filtrate used to inoculate 10 healthy cucumber plants, inoculations being made into stems and young leaves. No infection occurred on any of the plants thus inoculated.

(3) The remains of the vines present in the soil were removed, ground through a meat chopper, and enough sterile distilled water added to soak the material into a soft mass. Fragments of this were then inserted in incisions in the roots and stems of 10 healthy cucumber plants, but no infection resulted.

FIELD TESTS WITH CAGED PLANTS.

While this work gave only negative results, more conclusive series of experiments were conducted in the field during the summer seasons of 1915, 1916, and 1917. Observations during 1914 at Hamilton, Mich., where mosaic had been severe for some time, showed that the disease seemed to occur as early and severely on new land as on fields where mosaic cucumbers had been grown for two or three years in succession. An attempt was made therefore, to determine whether fields that had previously grown mosaic plants furnished a source of infection for crops the following season.

Hamilton, Mich., 1915.—One of the fields which had been under observation for two years was selected for work in 1915. The plat was one-eighth of an acre in size, had grown cucumbers for three consecutive years, and nearly every plant had been diseased each year. This plat was again planted to cucumbers in 1915, and as soon as the plants appeared 18 cheesecloth cages were distributed over it, each cage covering two plants. The cages were lifted only once during the season, to allow weeding and thinning, and every precaution was taken to avoid outside infection. Mosaic appeared in the plat about July 20, and on September 6 every uncaged plant showed mosaic symptoms. The cages were lifted on this date and the entire 36 plants were found free from disease.

Hamilton, Mich., 1916.—The same plat was used in 1916 and 30 cages set out on July 10, covering 60 plants. The uncaged plants became affected early in the season and on August 22 each of them showed the disease in severe form. The caged vines had grown so rapidly that it was necessary to remove the cages at this time, and it was found that all had remained healthy.

Big Rapids, Mich., 1916.—A test similar to that at Hamilton was conducted at Big Rapids, Mich., on a quarter-acre plat that had been badly diseased the previous year. Thirty-five cages were put down on June 20, five on June 29, and five on July 7. However, the disease was not severe in the uncaged portions of the field, and only 10 per cent of the plants were found to have mosaic at the end of the season, so that the results were not as conclusive as in the other tests. All caged plants remained healthy throughout the season.

Madison, Wis., 1917.—In 1917 the work was transferred to Madison, Wis, and the experiment was repeated on a ¾-acre plat where all plants had mosaic in 1916.

The cages in this case were set out on June 26, as soon as the seeds germinated, 18 cages being used, covering 36 plants. Beetles were present in the field in great numbers after July 10, and a few appeared in some of the cages after a heavy rain which had washed the earth away from the edges of the cages. The plants in these cages were removed on July 9 and new seed planted. The cages were watched carefully; no more insects appeared until August 18, when beetles were found working in nearly every cage, but as there seemed to be no means of entry from the outside it was suggested by entomologists who were consulted that they were from the second brood and had emerged from the soil. No effort was made to remove them, and the cages were left until September 13. The uncaged plants had been very badly infected with mosaic early in the season, 25 per cent of the plants developing the disease within five days after July 19, when the first cases of mosaic were found. By September 13, when all the uncaged plants had been infected for some time and the vines were much stunted, the caged plants still appeared perfectly healthy and normal with the exception of injuries from the beetles noted above. (Pl. IX, *B*.)

During the same season an additional test was carried on in a small garden belonging to the department of plant pathology. Here 12 cages were used to cover 24 vines which were transplanted from the greenhouse early in June. Fifty additional plants were transplanted near by and 100 more were grown from seed, both lots being left uncaged. All the uncaged vines became diseased by August 20, and when the cages were removed on August 29 all the caged plants proved perfectly normal and healthy.

The results of these experiments furnish very definite proof that the soil plays little, if any, part in the overwintering of cucumber mosaic. The fact that the virus has not been found in the tissues of old and decayed vines, coupled with the fact that vines protected from insects can be kept free from mosaic year after year on soils where the disease has been present for several consecutive seasons, seems to exclude the soil from consideration as a means of overwintering.

SEED AS A MEANS OF OVERWINTERING.

The problem of the transmission of mosaic diseases through the seed from infected plants has received much attention from various writers in the case of tobacco and tomato. No definite evidence of seed transmission in the case of tobacco has ever been obtained, although Allard (3) showed the presence of the virus in the flower parts, capsules, and mature seed. In the case of tomato mosaic, Miss Westerdijk (30) reported a possible example of the transmission of the infection by seed. Reddick and Stewart (24) have also stated that bean mosaic is seed borne. The question of seed transmission of certain mosaic diseases is therefore still an open one.

FIELD OBSERVATIONS.

Certain field observations on cucumber mosaic, together with the fact that the disease is prevalent in seed-growing districts, have made it of great importance to determine definitely whether the disease may be carried over on the seed each year.

As in the case of tobacco mosaic, the fruits of mosaic cucumber plants contain the infective principle, and their juices will produce infection. As far as can be determined, these juices remain infectious until the seeds are nearly mature, but the presence of the virus in the immature seed or in the tissues surrounding it can hardly be definitely proved, since the thick flesh surrounding the seeds makes it difficult to remove certain portions of the fruit, including traces of the juice from other cells. As stated on page 36, inoculations made from mature seed have never shown the presence of the virus, but it is possible that the seed may contain the virus in rare cases and perhaps afford an opportunity for overwintering the disease in this way.

The opportunity for seed infection is present, however, if such infection be possible, since the disease is prevalent in many of the seed-growing districts. Much of the cucumber seed, especially of pickling cucumbers, is grown in the vicinity of Muscatine, Iowa, Rocky Ford, Colo., and to some extent in Michigan and Ohio. The mosaic disease has been found to occur very commonly in these districts, and in handling the crop little or no effort is made to cull out fruits from mosaic plants. Since many of these fruits are nearly

normal in size and appearance at maturity, a great number are included with the seed crop each year and there is no doubt that much of the seed stock furnished to growers contains many seeds from mosaic vines.

During the last four years a number of cases of infection have been observed which gave some support to the theory of transmission of mosaic through the seed. At Hamilton, Mich., in 1914, careful field observations had been made during the first 10 days of July, and but one field was found in which mosaic was present. On July 12, however, at a distance of 4 miles from the town, a small patch of cucumbers was found in which 25 per cent of the plants were diseased. The plants were growing on land cleared the year previous and were surrounded by a belt of woods. No other cucumbers were within 2 miles, and the only mosaic plants found up to that time were 4 miles away. The plants showed no insect injury, and it was difficult to explain the origin of such early infection except by seed transmission.

Another striking case occurred at Big Rapids, Mich., in 1915. This locality was practically free from mosaic, and none was found until July 24. On this date one mosaic plant was found in the center of a 2-acre experimental plat of cucumbers. The land had never grown cucumbers before, and the few additional fields in the vicinity were free from the disease. The plant found was immediately covered with a cheesecloth cage, to prevent the spread of infection, and no further cases appeared during the summer with the exception of plants inoculated under cages.

Several other instances of infection occurring early in the season in isolated fields have been noted, and a few similar cases have appeared in the greenhouse, where the mosaic suddenly developed during the winter in houses that had previously been free from it and were so isolated that outside infection seemed unlikely.

McClintock (22) reported observations made at the Virginia Truck Experiment Station at Norfolk which he believes may indicate the transmission of cucumber mosaic by the seed. Plants from the greenhouse were transplanted to coldframes in the field, in some cases on sterilized soil. Considerable mosaic was later found on these plants, and no evidence of insects or insect injury was noted.

All field observations of the type mentioned are open to objection, however, in so far as they are taken as definite evidence of the transmission of the disease by the seed. So many sources of infection exist and their detection is often so difficult that infection may occur in isolated localities from sources that are easily overlooked. Insects, such as striped beetles, may travel considerable distances, especially in high winds, and might infect a few plants and disappear soon after. Mosaic plants of the wild cucumber (*Micrampelis lobata*) may also be present, being often overlooked because of their loca-

tion in out-of-the-way places. All such infection is so easily possible and so difficult to trace that field observations are always subject to doubt as proof of seed transmission of the disease.

TESTS WITH SEED FROM MOSAIC PLANTS.

In order to obtain more definite data in regard to seed transmission, a number of tests have been made with seed saved from mosaic plants. These trials were conducted both in the greenhouse and in the field, the field tests being at Big Rapids, Mich.

Tests of 1915.—Seed collected from mosaic plants at Hamilton, Mich., during the fall of 1914 was tested in the greenhouse of the department of botany at the Michigan Agricultural College at East Lansing, Mich., during the winter of 1914–15. The seed from individual plants was not kept separate in these trials, most of it being very immature, and only 90 plants were obtained from the entire lot. These were grown in pots on sterilized soil for five weeks, but no signs of mosaic developed.

Tests of 1916.—During the fall of 1915, seed was collected from mature fruits of mosaic cucumber plants, using only those fruits which actually showed evidence of the mottling and deformity characteristic of the disease. The seed from each fruit was removed separately, and in planting these separate lots were kept distinct.

A preliminary test was made during the winter of 1915–16 in the greenhouse at East Lansing, Mich., 500 plants being grown in pots on sterilized soil at a temperature of 28° C. (82° F.) and kept under close observation for seven weeks. No evidence of mosaic appeared on any of the plants during this trial.

An extensive field experiment was made during the summer of 1916 at Big Rapids, Mich., on land which had not grown cucumbers previously and in a district nearly free from the disease. The seed was from the lot used in the greenhouse test during the winter and represented seed collected from mosaic plants in Michigan, Indiana, Wisconsin, Iowa, and Ontario, Canada. The seeds from each fruit were planted in separate rows 3 feet apart, with the seeds about 4 to 8 inches apart in the row. Approximately 100 fruits were represented, which gave about 4,500 plants for observation. The germination was very uneven, but this seemed to result from the immaturity of some of the seed rather than from any effect of the disease, since all seed which germinated produced plants of healthy appearance. Observations were made practically every day in the early part of the season and at least once every two days in August and September. All plants remained healthy and normal in appearance and no disease appeared in the adjoining plats until July 28. On this date mosaic appeared on uncaged plants in a plat close to the seed test, through the accidental escape of aphids used in other experiments. In spite

of drastic efforts for its eradication, the disease spread gradually through the plat until it reached the rows adjoining the seed test, where it first appeared on August 8. After this date, it worked slowly through the plat, although all mosaic vines were removed as soon as noted. The plants had remained healthy for seven weeks, however, and the disease which appeared was so clearly a result of outside infection that the evidence of seed transmission may safely be called negative.

Tests of 1917.—In 1917 a further trial of mosaic seed was again conducted at Big Rapids, Mich., using seed collected from Michigan, Indiana, and Wisconsin. In this work the seed from 119 fruits was planted on new land, located at some distance from that used in former experiments and on considerably higher ground.

The plat was about 1 acre in size and was between two other blocks of cucurbits, principally cucumbers. No other experiments with mosaic were made during the season in any of the plats. About 5,500 plants were under observation and 1,150 of these were covered with cheesecloth cages to prevent accidental infection by insects. Use was made of 250 cages, each covering from 3 to 15 plants. They were set out as soon as the seed was planted and were not lifted until late in July, except where repairs were necessary. The writer was absent from Big Rapids most of the time, and the planting and early inspection work was done under the supervision of Mr. W. W. Gilbert. The plants were left unthinned as long as possible and were inspected at frequent intervals. The writer examined the plants, beginning July 30, and lifted all cages for the purpose of inspection. Great care was taken to avoid outside infection from insects, and the cages were replaced at once. A few striped beetles were present in the field, but only one cage was found to contain insects. All uncaged plants in the seed test and in the adjoining plats were free from mosaic, and all caged plants were healthy with a single exception. This plant was abnormally dwarfed, its lower leaf was yellow and wilted, and the four other leaves were peculiarly dark green in color and showed slight indications of a yellow mottling. (Pl. I, *A*.) All of the remaining plants which were in the same cage were of normal size and appearance, without the least trace of insect injury. This cage had no defects in its covering and had not been lifted after the seed was planted.

The writer again visited the field on August 18 and inspected the plants. No case of mosaic was found in the plat with the exception of the suspected plant found on July 30. The appearance of this was so unmistakably mosaic that 12 healthy plants were inoculated from the juice of one of its leaves and covered with cages. Most of these developed typical mosaic symptoms on leaves and fruits, and there is no doubt that the plant in question was mosaic. Mr. Gilbert reported that no further cases of mosaic appeared on cucumbers in

any plat during the season, but two plants of Hybrid Casaba melon in another plat did later develop typical mosaic symptoms. These plants were pulled as soon as found, and no other cases occurred. The conditions at Big Rapids during 1917 were unusually favorable for a test of this kind, since the district was practically free from mosaic, and insects were not unusually numerous. The single case of mosaic that occurred in the seed test can hardly be attributed to outside agencies, since soil and insects were eliminated almost beyond question and there was apparently no source of infection in the district adjacent to the experimental field. Furthermore, the very nature of the disease symptoms was such as might be expected in cases of seedling infection, the plant being much dwarfed and the first leaf showing signs of having been affected almost as soon as it appeared.

Another case has occurred which is very similar to the above and lends support to the theory of seed as a means of overwintering. Seed was planted in the greenhouses of the plant-pathology department of the University of Wisconsin in the fall of 1917, immediately after the houses had been thoroughly cleaned and fumigated and fresh soil placed in the benches. The planting was made on September 12, using ordinary commercial cucumber seed, and the plants were under observation daily after they appeared. On September 23, one plant out of 65 showed definite mosiac infection, the cotyledons were yellowed and wilted, and the first true leaf, which was very small, was mottled and distorted. (Pl. X, *A*.) The plant was proved to be infected by mosiac by the successful inoculation of several healthy plants from it. It was kept for several weeks in a separate house for observation and later developed the same peculiar wrinkling and yellowing observed in the case at Big Rapids. It remained much stunted and deformed. All the other plants grew vigorously and showed no signs of the disease. (Pl. X, *B*.)

In this case the factor of insect transmission can not be entirely eliminated, since insects were present in the near-by fields until some time in November, but the facts that no injury was visible on any plant in the house and no insects could be found partly removed this objection, since slight injury of this kind is usually very noticeable on young seedlings.

The results so far, therefore, show that out of 10,000 plants grown from seed from mosiac vines, only one has developed a case of mosaic that could not definitely be attributed to outside infection. Together with this is the case above noted, which appeared in the greenhouse under conditions that rendered outside infection very unlikely. The single case of infection which appeared in the open field at Big Rapids, Mich., in 1915, is most easily explained on the basis of seed transmission, since no other infection developed in the field aside from that resulting from artificial inoculation. The field data aside from this

are of rather doubtful value as evidence of the occurrence of the disease from the seed. While these data seem overwhelmingly negative at first glance, the single case of mosaic which developed from the seed of mosaic vines was of such a character that it is difficult to explain its appearance on any basis other than that of seed transmission. It is doubtful whether cucumber mosaic is seed borne to any considerable extent, but it seems possible that it may occur in rare cases, and the results so far obtained have left the problem still open, warranting further investigation. In general, the disease appears every year in most infested districts, and in a manner that would preclude any belief in seed transmission as the sole cause of its outbreak. In districts where the disease had not appeared, however, a single case of seed transmission in several thousand plants would be sufficient to introduce the disease, and the many agents of dissemination present would insure its rapid spread to other plants and fields.

INSECTS IN RELATION TO OVERWINTERING.

The amount of cucumber mosaic present each year in various localities has frequently been in direct relation to the number of cucumber insects in the fields. This would be expected in view of their importance as agents of dissemination. It has been found, also, that the first appearance of the disease in many localities seems closely related to the number of insects present, as far as the amount of the early infection is concerned. During 1916 and 1917 there was an unusually small number of cucumber insects in southwestern Michigan, and the disease appeared in a much less severe form than in previous seasons. During the same period, cucumber insects at Madison, Wis., and in adjacent localities were present in unusually large numbers in June and July, particularly the Diabrotica species. The disease developed there with great severity both years, several fields in 1917 having 15 to 25 per cent of the plants affected within five days from the time the first case was noted. These observations led to the belief that insects might bear some part in the overwintering of mosaic.

THE RELATION OF APHIDS TO OVERWINTERING.

During 1915 and 1916 an attempt was made to overwinter aphids taken from mosaic vines, but no insects appeared in the spring and, as little is known of the method by which this insect passes the winter, the work was finally abandoned. As the cucumber aphis (*Aphis gossypii*) has always appeared rather late in the season and not until some time after the disease developed, it is probable that this insect is not a factor in the first appearance of the disease. This is further indicated by the fact that aphids are not present every year in localities where the disease appears each season.

THE RELATION OF STRIPED CUCUMBER BEETLES TO OVERWINTERING.

The work of Rand (23) on the Diabrotica species as possible carriers of the bacterial wilt organism (*Bacillus tracheiphilus* Smith) has already shown that these insects are an important factor in the overwintering of one of the diseases of cucurbits and strengthened the hypothesis that they might bear the same relation to mosaic overwintering. The striped cucumber beetle (*Diabrotica vittata*) and the 12-spotted beetle (*D. duodecimpunctata*) both overwinter in the adult stage, emerging from hibernation in the spring, and thus offer a more definite possibility of overwintering the disease than the aphis, so that the later experiments have all been with the Diabrotica species.

Artificial hibernation experiments.—The work with beetles was done by the writer in cooperation with Mr. Neale F. Howard, of the Bureau of Entomology, United States Department of Agriculture. Mr. Howard took entire charge of the collection and caging of the beetles and of all work pertaining to the actual hibernation and life history of the insects, and the writer conducted the tests relative to disease transmission by these insects.

In the fall of 1916 some 8,000 beetles, chiefly *Diabrotica vittata*, were collected from fields about Madison, Wis., where mosaic was prevalent. The beetles were placed in hibernation cages of various sizes and types (Pl. IX, *B*) and fed on mosaic cucumber vines and fruits as long as such material was available. Most of the cages were kept outdoors in various places during the winter, a few being in more sheltered locations than others. The base of each cage contained from 1 to 3 feet of soil and trash, to furnish favorable conditions for hibernation. The number of insects varied with the size of the cage, but at least 200 were placed in each cage. During the winter some of the smaller cages were brought into the greenhouse at intervals and observed for the possible appearance of the beetles. Only three emerged during the winter, however, and no infection resulted when they were placed on healthy cucumber plants. Aside from these few insects, none appeared from any of the cages either in the early spring or in June and July, so that no data on beetle overwintering of the disease was obtained from these tests.

During the fall of 1917 these tests were repeated at Madison, Wis., on a larger scale, about 35,000 insects, both *Diabrotica vittata* and *Diabrotica duodecimpunctata*, being used. The beetles were collected during September and October and placed in various outdoor locations, most of the cages being near points where beetles had appeared early that spring. Fifteen cages were used in these experiments, each having from 500 to 5,000 insects, most of them containing from 2,000 to 3,000. The cages were usually made with the base extending deeply into the soil but open at the bottom; the soil

within the cage was not disturbed and the natural covering of grass and leaves was left intact. The insects were fed on the fruits and leaves of mosaic cucumber plants as long as these could be secured. Later in the fall seeds of the wild cucumber (*Micrampelis lobata*) were scattered in all the cages, in order to furnish plants for an immediate test of the beetles when they emerged in the spring.

On June 17, 1918, two beetles appeared in each of two cages, but no others were found in any cages during the rest of the season. The beetles which appeared were allowed to feed on the wild cucumber plants present in the cages, but were not otherwise tested. The writer did not observe these plants after June 22, but other observers report that no mosaic appeared. So far, therefore, the tests of beetles hibernated under artificial conditions have given no evidence that the insects are concerned in the overwintering of the disease.

Natural hibernation experiments.—Frequent observations were made in the fields at Madison, Wis., during the spring of 1917, and the first insects which appeared were collected and tested for mosaic infection. The first beetles were noted on May 25, the insects suddenly appearing on seedlings of *Micrampelis lobata* growing in a sand pit about 50 yards from the main experimental plats of 1916 and 1917. About 250 of these beetles were collected by the writer between May 25 and June 5, and varying numbers of them were placed on healthy cucumber plants under cages in the greenhouse. In this work 83 plants were used, and the insects were allowed to remain on them for two weeks, but no mosaic infection occurred. In addition to these tests, 60 small cucumber plants were set out in the sand pit and in adjacent fields at points where they would be likely to attract the beetles. Nearly all these plants were somewhat injured by the insects, but all remained healthy until after mosaic had developed at other points in the locality.

The first beetles to appear in the spring of 1918 were again tested in the same manner as in 1917. About 400 striped beetles were collected between May 15, when they first appeared, and May 28. These were placed under cages with healthy cucumber plants in the greenhouse, 98 plants being used for the tests. About 100 beetles were also collected at Plymouth, Ind., during the latter part of May and similarly tested on 25 healthy cucumber plants. No mosaic appeared on any of these plants, however, although they were observed for three weeks. Young plants were set out in the sand pit as in 1917, but no signs of mosaic developed, although the plants were exposed to the beetles from May 30 to June 20.

Field observations in 1917.—The striped beetles were very numerous about Madison, Wis., during June and July, 1917, and the young cucumber plants, which appeared about June 25 on the experimental plats, were severely injured by them. No mosaic was found in any

field until July 19, although all plats were carefully inspected at intervals of two to four days. On July 19 two plats out of six within a radius of a mile developed a few cases of mosaic, and six days later 25 to 30 per cent of the plants in both plats showed symptoms of the disease. The remaining fields also showed from 5 to 15 per cent of mosaic plants on that date. All cases were of the same age and seemed to have developed almost simultaneously. In view of the fact that insects had been attacking the plants for four weeks previous to the appearance of the disease, while the period of incubation is normally 7 to 12 days, it is difficult to explain such a sudden and extensive outbreak of the disease on the basis of insect overwintering and transmission.

That seedling plants may be infected when small but show no signs of the disease until they reach a certain period in their development has been considered a possible means of connecting such sudden outbreaks of the disease with insect agencies. No such abnormally long incubation period has ever been noted, although at least 200 seedlings have been kept under observation for several weeks after inoculation. Secondary inoculations from such plants have never produced the disease except in cases where there were definite symptoms of mosaic in the original plant. There is no evidence, therefore, that such a prolonged incubation period does occur.

Further evidence against the theory that insects carry the disease over winter is also furnished by the results of the work on disease transmission during 1917. As shown in Table XVIII, the beetles collected after frost had killed the vines failed to induce the disease when placed in cages with healthy plants, while all beetles collected earlier in the season contained some individuals which were carriers of mosaic. It is quite probable, therefore, that only a very small percentage, if any, of the beetles which go into hibernation carry the disease, since they are present in the fields for some time after all mosaic vines have been killed by frost. In view of the results obtained in the artificial hibernation studies, it is likely that only a few of those beetles which go into hibernation survive the winter, and field observations also indicate that only a small percentage of the insects present in the fields in the fall appear in the spring. If this is the case, a large number of insects would necessarily have to be tested to insure finding individuals which carry the disease, if ever any such are present. Rand (23) has found that only a small number of the striped beetles which survive the winter are carriers of *Bacillus tracheiphilus*, and this is even more likely to be the case with cucumber mosaic. The number of beetles so far tested is too few, perhaps, to warrant definite conclusions, but the evidence to date does not lend much support to the theory that insects are instrumental in carrying the disease over winter.

MICRAMPELIS LOBATA AS A SOURCE OF EARLY INFECTION.

As has already been stated, there are two wild species of the Cucurbitaceæ which are native in the Middle West. These are *Micrampelis lobata* and *Sicyos angulatus*. The latter does not occur as commonly in Michigan and Wisconsin as *Micrampelis lobata*, which is found in many localities where cucumbers are grown, either wild or used as an ornamental vine. Observations in Michigan showed that the micrampelis was subject to a mosaic disease similar to that on cucumber, cases being observed during 1915 and 1916 by Dr. E. A. Bessey, Mr. W. W. Gilbert, and the writer. Inoculations made from mosaic cucumber to healthy plants of micrampelis in the spring of 1917 resulted in typical cases of mosaic, and reciprocal inoculations from mosaic micrampelis to cucumber were equally successful, as shown in Table XX, thus proving the identity of the two diseases.

TABLE XX.—*Results of cross-inoculations from mosaic cucumber plants to healthy plants of Micrampelis lobata and from mosaic micrampelis to healthy cucumber plants.*

INOCULATIONS FROM MOSAIC CUCUMBER PLANTS TO HEALTHY PLANTS OF MICRAMPELIS.

Date inoculated.	Treatment.	Inoculum.	Source of infection.	Number of plants inoculated.	Results.	
					Number of mosaic plants.	Date observed.
May 11, 1917	Inoculated	Crushed tissue of mosaic leaf.	Artificial infection.	4	1	May 22, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	4	0	Do.
May 16, 1917	Inoculated	Crushed tissue of mosaic leaf.	Artificial infection.	5	2	May 29, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	5	0	Do.
May 24, 1917	Inoculated	Crushed tissue of mosaic leaf.	Artificial infection.	9	7	June 10, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	9	0	Do.
June 20, 1917	Inoculated	Crushed tissue of mosaic leaf.	Artificial infection.	4	2	July 3, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	4	0	Do.
Aug. 31, 1917	Inoculated	Crushed tissue of mosaic leaf.	Natural infection.	1	1	Sept. 10, 1917
Sept. 3, 1917	do	do	do	6	4	Sept. 12, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	6	0	Do.

INOCULATIONS FROM MOSAIC MICRAMPELIS PLANTS TO HEALTHY CUCUMBER PLANTS.

May 24, 1917	Inoculated	Crushed tissue of mosaic leaf.	Inoculated from mosaic cucumber plant.	10	9	June 9, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	10	0	Do.
July 3, 1917	Inoculated	Crushed tissue of mosaic leaf.	Natural infection.	8	6	July 15, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	7	0	Do.
Oct. 8, 1917	Inoculated	Crushed tissue of mosaic leaf.	Natural infection.	6	2	Oct. 14, 1917
Do	Control	Crushed tissue of healthy leaf.	.....	6	0	Oct. 20, 1917
Oct. 30, 1917	Inoculated	Expressed juice of mosaic plant.	Natural infection.	10	7	Nov. 10, 1917
Do	Control	Expressed juice of healthy plant.	.....	8	0	Do.

The fact that the micrampelis plant is so common, coupled with the presence of occasional mosaic infection, made it seem possible that it might be a source of spring infection to cucumber. During 1917 a close inspection was made of all plants which could be found in the western portion of Madison, Wis., and in the country adjoining the fields where the cucumber plats were located. A particularly large number of micrampelis plants were found in the sand pit already referred to near the main experimental field. On July 9 eight plants in two groups were found at this point, all of them showing typical mosaic symptoms. No mosaic was found on any other cucurbit host until at least 10 days later.

Mr. I. C. Hoffman, working at Plymouth, Ind., found several wild micrampelis plants which showed symptoms of mosaic as early as June 30. The plants in both cases were proved to be truly mosaic by successful inoculations on cucumber.

No other cases were found prior to the appearance of the mosaic disease on the cultivated host in the spring of 1917, but during the summer 10 different centers of mosaic micrampelis were found in the vicinity of Madison at rather widely separated points. In the fall of 1917 the disease was also reported by Mr. W. W. Gilbert as occurring at Big Rapids, Mich., and Brighton, Colo., and by Mr. I. C. Hoffman at Fort Collins, Colo.

Observations were again made about Madison during the spring of 1918, particularly in localities where the disease had appeared on micrampelis in 1917. In two such locations the disease was again found on June 15. In one case 4 out of 18 plants were infected with mosaic and in the other case 6 out of 27 showed it. In all cases the plants were about 5 weeks old. Cultivated cucurbits were being planted at that time, and none of the adjacent gardens contained any cucurbits of other species. Several mosaic micrampelis vines were also found by Mr. W. W. Gilbert at Big Rapids, Mich., on June 23, 1918. These plants were being grown as ornamentals near a garden where the disease had been found the previous season on cucumbers. They were 4 to 6 feet tall when seen, while the cucumbers in near-by gardens were only a few inches high and in the adjacent fields few were up. A considerable number of striped beetles were observed feeding on the micrampelis vines.

There is no doubt, therefore, that the disease occurs on wild plants some time before its appearance on cultivated hosts, since most cucumber fields had just been planted at the time the disease appeared on the micrampelis vines. The source of infection on the micrampelis plants in these cases was undetermined. To test the possibility of seed overwintering, about 150 plants were grown from seed collected in each of the Madison localities in 1917. The plants were grown in the greenhouse for six weeks during January and Feb-

ruary, 1918, in connection with the other test of seed from mosaic micrampelis mentioned below. No mosaic symptoms appeared on any of the plants. No mosaic was present in the greenhouses in the vicinity of Madison, and while striped beetles attacked the vines, their importance in overwintering the disease is unknown.

Wild micrampelis plants are common in all parts of Michigan and Wisconsin, and in addition to their occurrence in nature the vine is often planted where a quick-growing ornamental is desired. After being once planted it usually continues to appear each year from self-sown seed.

The presence of mosaic on *Micrampelis lobata* in advance of its appearance on any cultivated cucurbit host, coupled with the fact that the striped beetles feed upon micrampelis as soon as they emerge from hibernation and go directly from the wild plant to the cultivated cucumber when the latter appears, offers a most promising explanation for the early infections of the cucumber. There is little doubt, apparently, that *Micrampelis lobata* may be a source of such early infection to the cucumber.

The source of primary infection on the wild plant is still uncertain, but much of the work on the overwintering of the disease on the cultivated cucumber seems equally applicable to the wild species. Soil is probably not a factor and the question of seed as a means of transmission is still unsettled, although the appearance of the disease in the same spot each year would indicate that the seed may be the means of overwintering. During the winter of 1917–18, 1,100 plants were grown in the greenhouse from seed collected from mosaic micrampelis plants in Michigan, Indiana, Wisconsin, and Colorado. An additional lot of 1,000 plants was also grown from seed taken from plants supposedly free from mosaic. The conditions under which the plants were grown were unfavorable, however, and caused the leaves to develop various abnormal colorings, which made it difficult to determine whether any of the plants were infected with mosaic. Inoculations from suspected plants gave negative results in all cases, but further tests of seed from mosaic plants are in progress under more favorable conditions for observation.[1]

WILD NONCUCURBITACEOUS HOSTS.

The work of Jagger (19), already mentioned, has shown that the mosaic disease of cucurbits may be transferred to some plants outside the Cucurbitaceæ. Although there is no definite evidence that the disease overwinters on plants of other families, it is possible that such wild hosts, particularly if they are perennial, might serve as sources of early infection to the cucurbits, especially as cucumber insects are often found feeding on plants of other families. All cross inocula-

[1] Subsequent tests have proved that the mosaic disease may be carried over winter in the seed of *Micrampelis lobata*. See Phytopathology, vol. 9, p. 326, 1919.

tions made by the writer from plants outside the Cucurbitaceæ have given negative results, but the large number of wild plants which show various types of chlorotic diseases makes the problem one which will require much inoculation work before final conclusions are justified.

CONTROL MEASURES.

All efforts to control the mosaic of cucurbits have dealt with cucumbers in the field and have been along three lines: (1) The removal of diseased plants as soon as they are detected, (2) the control of insects which spread the disease, and (3) the breeding of a cucumber which would be resistant to the mosaic disease.

SANITARY MEASURES.

The tests of sanitary measures were made during the season of 1916, a considerable acreage of land being available for this purpose at Plymouth, Ind., Madison, Wis., and Big Rapids, Mich. The absence of the disease from all but one field at Big Rapids limited the work to a single plat of about one-third of an acre, where aphids from mosaic experiments escaped by accident and furnished a severe infection. The disease appeared in the outside row of the plat on July 28, the aphids being by that time well disseminated on the rows closely adjoining. All plants in this row were pulled and burned where they lay, the remainder of the field was sprayed with nicotine sulphate, and from that date to the end of the season plants in the plat were inspected daily and all those suspected of mosaic were removed and destroyed. Once started, however, the disease continued to increase, and by August 25 the number of plants had been reduced about 50 per cent. The removal of diseased vines after they had become intertwined with healthy plants resulted in contact infection of those adjacent and served to increase rather than diminish the disease.

The work at Madison, Wis., was under the direction of Dr. M. W. Gardner during 1916 and included a large acreage of cucumbers. The disease developed rapidly after its first appearance, due probably to the presence of an abnormal number of cucumber insects, and attempts at removing all mosaic plants soon become hopeless, many fields being practically ruined by August 9. Similar results were secured in the experiments at Plymouth, Ind., which were conducted by Dr. George A. Osner.

The fact that diseased plants are a source of infection before definite symptoms appear, which had not been proved at that time, adds to the difficulty of control by sanitary measures. While the eradication method is theoretically sound, the practical conditions in the field are such that, except for use early in the season and in the case of slight infections in isolated fields, this method affords little hope of success in stopping the progress of the disease.

INSECT CONTROL.

The attempts at insect control have been under the direction of Mr. Neale F. Howard, of the Bureau of Entomology. This work was conducted at Madison, Wis., and Plymouth, Ind., and is still in progress. It is possible to control insects to such a degree as will prevent severe injury to the plants, but complete insect elimination is necessary to prevent disease transmission by them, and methods for accomplishing this have not yet been worked out. In the greenhouse, insect elimination is more easily possible, and if the grower is well acquainted with mosaic he might succeed in eradicating or at least so checking the disease that little loss would occur. In the field, however, some further knowledge of the method of overwintering of the disease and the cause of its sudden appearance in the spring is necessary before adequate methods of control can be devised.

RESISTANT VARIETIES.

Attempts have been made to obtain a variety of cucumber resistant to the mosaic disease, but so far this work has been unsuccessful. Selections made in a mosaic field at Muscatine, Iowa, in 1915 by Mr. W. W. Gilbert were tested at Holland, Mich., in 1916, but the amount of infection present in the field was not sufficient for a fair trial. Selections from this strain which gave some promise of resistance were again planted at Madison, Wis., in 1917, but all the plants developed mosaic symptoms early in the season. During the past four seasons the writer has never observed any indication of resistance in any variety of cucumbers, and the fact that little evidence of resistance has appeared in the case of other mosaic diseases indicates that the development of a resistant variety is likely to be difficult, if at all possible.

SUMMARY.

The mosaic disease of cucurbits has apparently been present in the United States for nearly 20 years, but prior to 1914 its importance was practically unrecognized.

The disease appears both in the field and in the greenhouse in nearly all sections where cucurbits are of commercial importance. Nearly all cultivated cucurbits are susceptible to the disease, but the cucumber crop seems to be most seriously affected, particularly in the Central States and the trucking regions of the South.

The diseased plants develop a yellow mottling of the younger leaves, accompanied by a wrinkled or savoyed appearance. The older leaves gradually turn yellow and die, leaving the basal portion of the stem bare. These bare stems terminate in a rosettelike cluster of dwarfed leaves, which lie close to the ground, owing to the

shortening of the petioles and internodes of the stem. Mosaic fruits of the cucumber are mottled with green and yellow and often develop dark-green wartlike outgrowths. The Summer Crookneck squash also shows a mottled and warted appearance, but the fruits of most other cucurbits are little changed.

Nearly all species and varieties of the genera Cucumis, Cucurbita, Lagenaria, Luffa, Momordica, Trichosanthes, Ecballium, Benincasa, Micrampelis, and Sicyos are susceptible to the disease, but the Citrullus species seem to be partially resistant.

The pathologic anatomy of the mosaic leaves shows a distinct variation from the normal in the tissues of the mottled leaves. The palisade cells of the green portions of such leaves are longer and narrower than similar cells in the yellow portions. The spongy parenchyma is more compact in the yellowed areas, and the chloroplasts are somewhat smaller than those of the green portions of the leaf. Similar differences appear in the cells directly below the epidermis in mottled cucumber fruits, but all the other tissues of the fruit are normal in appearance. The structure of the stems and roots of mosaic plants does not differ from that of healthy plants.

No visible causal organism has been associated with cucurbit mosaic, and the disease appears to be unrelated to soil conditions. The juice of mosaic plants contains an infective principle, or virus, however, which possesses certain definite properties.

The expressed juice of mosaic plants is rendered noninfectious if heated above 70° C. The power of infection is also destroyed by formaldehyde, phenol, and copper sulphate in 0.5 per cent solutions and by mercuric chlorid in a strength of 1:2,000. A 10 per cent solution of chloroform will also render the virus inactive, but neither 5 per cent chloroform nor 10 per cent toluene are effective.

The juice of mosaic diseased plants may be diluted to 1:10,000 and still retain the power of infection. Filtration of the expressed juice of mosaic plants through a Berkefeld filter does not remove this power of infection, but Chamberland filters have rendered the filtrate noninfectious. The expressed juice of mosaic plants rarely remains infectious longer than 24 to 48 hours, and the virus is rapidly destroyed by desiccation.

The infective principle, as far as it has been determined, possesses many properties of a living organism, and it appears possible that the disease may be caused by an ultramicroscopic parasite.

The mosaic is highly infectious and can be produced by introducing the expressed juices or crushed tissues of a mosaic plant into slight wounds in healthy plants. Inoculations may be made at any point in the stem or leaf, including the leaf trichomes. Infection can also be produced through the fruit, but has never resulted when

the virus was introduced into the roots or flower parts. The first symptoms of the disease invariably appear in the youngest leaves or fruits and the susceptibility of the plant seems to be closely related to its age and vigor of growth.

The virus spreads through the plant from the point of inoculation and is present throughout the leaves and stems 24 to 48 hours before any visible symptoms appear. There is some evidence that the vascular system may be the chief channel of distribution in the plant.

Transmission under field conditions in the case of the cucumber may occur during the thinning, training, or picking operations and also by the removal of diseased vines which are intertwined with healthy plants. Cucumber insects are among the most important agents in the transmission of the disease in both the field and the greenhouse. The melon aphis (*Aphis gossypii* Glover) and the striped and 12-spotted cucumber beetles (*Diabrotica vittata* Fabr. and *Diabrotica duodecimpunctata* Oliv.) are the insects most concerned. Bees have not been proved to be carriers of the disease.

It has been shown that the disease does not live over winter in the soil, and there is no evidence that striped cucumber beetles or other insects are a source of primary infection in the spring. Extensive field tests of seed from mosaic plants and observations in the field and greenhouse indicate that infection through the seed may possibly occur in rare cases.

The mosaic diseases of tobacco, tomato, bean, potato, pokeweed, and various other hosts do not appear to affect the cucumber. Inoculations of these and other plants outside the Cucurbitaceæ (except martynia, see p. 6) with the expressed juice of mosaic cucurbits have also given negative results.

The wild cucumber (*Micrampelis lobata*) is affected with a mosaic disease identical with that on the cucumber. Diseased plants of this species have been found in Wisconsin and Indiana at least two or more weeks before the disease appeared on the cultivated cucumber. The striped cucumber beetles feed on the micrampelis from the time they appear and later go directly from the wild plants to cultivated cucumbers. There is thus a direct means of transmission from the wild to the cultivated host during the early part of the season.

The source of primary infection of micrampelis is not certain, the factors which appear to have been eliminated in the case of the cultivated cucumber probably being excluded from the wild host also. The wild host plant, however, offers the most definite source yet discovered of primary infection for the cucumber.

The control methods thus far tested have been (1) the removal of diseased plants as soon as found; (2) the control of cucumber insects, which are largely instrumental in spreading the disease; (3) the discovery of varieties of cucumbers which are resistant to mosaic; and

(4) the caging of plants to keep insects away. None of these methods has proved entirely effective and practicable under field conditions.

In the field, removal of diseased plants has proved of little value except for the first cases early in the season, because they may be sources of infection for one to three days before they are found. Even with the best insect-control methods available, enough beetles are left to cause a wide dissemination of mosaic. All efforts at finding strains of cucumbers resistant to the disease have given negative results. The use of cages is practicable for a few plants in the home garden, but not feasible on larger areas.

Under greenhouse conditions the elimination of insects and the removal of mosaic plants are possible, and these control measures have proved of great value.

Satisfactory control measures for field conditions will necessarily have to await the discovery of more definite data regarding the sources of primary infection.

The importance of wild host plants as a means of overwintering the mosaic disease, the possibilities of infection through seeds from diseased plants, and the relation of insects to overwintering must be studied further before effective control recommendations can be made.

LITERATURE CITED.

ALLARD, H. A.

(1) 1914. The mosaic disease of tobacco. U. S. Dept. Agr. Bul. 40, 33 p., 7 pl.

(2) 1915. Effect of dilution on the infectivity of the virus of the mosaic disease of tobacco. *In* Jour. Agr. Research, v. 3, no. 4, p. 295–299.

(3) 1915. Distribution of the virus of the mosaic disease in capsules, filaments, anthers, and pistils of affected tobacco plants. *In* Jour. Agr. Research, v. 5, no. 6, p. 251–256, pl. 23.

(4) 1916. Some properties of the virus of the mosaic disease of tobacco. *In* Jour. Agr. Research, v. 6, no. 17, p. 649–674, pl. 91. Literature cited, p. 673–674.

(5) 1917. Further studies of the mosaic disease of tobacco. *In* Jour. Agr. Research, v. 10, no. 12, p. 615–632, pl. 63.

(6) BEIJERINCK, M. W.

1898. Ueber ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verhandel. K. Akad. Wetensch. Amsterdam, sect. 2, deel 6, no. 5, 22 p., 2 col. pl.

(7) CHAPMAN, G. H.

1917. Mosaic disease of tobacco. Mass. Agr. Exp. Sta. Bul. 175, p. 73–117, 5 pl.

CLINTON, G. P.

(8) 1908. Notes on fungus diseases etc., for 1908. Muskmelon chlorosis. *In* Conn. Agr. Exp. Sta. Ann. Rpt. [1906]/08, p. 865–866.

(9) 1916. Mosaic, or white pickle. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1915, p. 430–431, pl. 19.

(10) COONS, G. H.

1915. Notes on plant diseases of Michigan. Cucumber mosaic, or white pickle. 17th Rept. Mich. Acad. Science., p. 125–126, 1 pl.

DOOLITTLE, S. P.

(11) 1916. A new infectious mosaic disease of cucumber. *In* Phytopathology, v. 6, no. 2, p. 145–147.

(12) ——— and GILBERT, W. W.

1918. Further notes on cucumber mosaic disease. (Abstract.) *In* Phytopathology, v. 8, no. 2, p. 77–78.

(13) FREIBERG, G. W.

1917. Studies in the mosaic diseases of plants. *In* Ann. Mo. Bot. Gard., v. 4, p. 175–232, pl. 14–17. Bibliography, p. 223–225.

(14) GILBERT, W. W.

1916. Cucumber mosaic disease. *In* Phytopathology, v. 6, no. 2, p. 143–144, pl. 5.

(15) HUNGER, F. W. T.

1905. Untersuchungen und Betrachtungen über die Mosaikkrankheit der Tabakspflanze. *In* Ztschr. Pflanzenkrank., Bd. 15, Heft 5, p. 257–311, 1 fig. Literatur, p. 309–311.

(16) IWANOWSKI, D.

1903. Über die Mosaikkrankheit der Tabakspflanze. *In* Ztschr. Pflanzenkrank., Bd. 13, Heft 1, p. 1–41, pl. 1–3.

JAGGER, I. C.

(17) 1916. Experiments with the cucumber mosaic disease. *In* Phytopathology, v. 6, no. 2, p. 148–151.

(18) 1917. Two transmissible mosaic diseases of cucumber. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 61.

(19) 1918. Hosts of the white pickle mosaic disease of cucumber. *In* Phytopathology, v. 8, no. 1, p. 32–33.

(20) 1918. Mosaic disease of cucurbits. (Abstract.) *In* Phytopathology, v. 8, no. 2, p. 74–75.

(21) KONING, C. J.

1899. Die Flecken- oder Mosaikkrankheit des hollandischen Tabaks. *In* Ztschr. Pflanzenkrank., Bd. 9, Heft 2, p. 65–80, 2 fig.

(22) MCCLINTOCK, J. A.

1916. Is cucumber mosaic carried by seed? *In* Science, n. s., v. 44, no. 1144, p. 786–787.

(23) RAND, FREDERICK V.

1915. Dissemination of bacterial wilt of cucurbits. (Preliminary note.) *In* Jour. Agr. Research, v. 5, no. 6, p. 257–260, pl. 24.

(24) REDDICK, DONALD, and STEWART, V. B.

1918. Varieties of beans susceptible to mosaic. *In* Phytopathology, v. 8, no. 10, p. 530–534.

(25) RUGGLES, A. G., and STAKMAN, E. C.

1911. Orchard and garden spraying. Minn. Agr. Exp. Sta. Bul. 121, 32 p.

SELBY, A. D.

(26) 1903. Report of committee on vegetable pathology. A peculiar malady of forced cucumbers. *In* Ann. Rpt. Ohio State Hort. Soc., 1902, p. 109.

(27) 1910. A brief handbook of the diseases of cultivated plants in Ohio. Ohio Agr. Exp. Sta. Bul. 214, p. 307–456, 106 fig. Literature, vii p.

(28) STAKMAN, E. C., and TOLAAS, A. G.

1916. Fruit and vegetable diseases and their control. Minn. Agr. Exp. Sta. Bul. 153, 67 p., 32 fig.

(29) STONE, G. E.

1910. Calico or mosaic disease of cucumber and melon. *In* Mass. Agr. Exp. Sta. 22d Ann. Rpt. [1909], pt. 1, p. 163.

(30) WESTERDIJK, JOHANNA.

1910. Die Mosaikkrankheit der Tomaten. Meded. Phytopath. Lab. "Willie Commelin Scholten," Amsterdam, 1, 19 p., 3 pl. (1 col.).

WOODS, A. F.

(31) 1900. Stigmonose. A disease of carnations and other pinks. U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 19, 30 p., 5 fig., 3 pl. (1 col.).

(32) 1902. Observations on the mosaic disease of tobacco. U. S. Dept. Agr., Bur. Plant Indus. Bul. 18, 24 p., 6 pl. (3 col.).

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
15 CENTS PER COPY